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THREE PREVIOUSLY UNDESCRIBED MOSAIC DISEASES OF PEA[1]

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INTRODUCTION

During the last few years a number of distinct viruses affecting pea (*Pisum sativum* L.) have been named and described. In the course of experimental studies on varietal susceptibility and resistance of peas to a virus from mosaic-infected alsike clover, the writer found three other viruses on peas which produced symptoms somewhat unlike those already described on certain varieties. Studies were then conducted to determine the symptoms produced by these viruses on peas and beans and the resistance and susceptibility of a number of varieties of each. Host range and physical properties of the viruses in vitro were also investigated. These studies disclosed sufficient differences to permit classification and identification. A comparative study of these viruses and of an earlier described mosaic virus from alsike clover likewise infectious to pea is presented in this paper.

REVIEW OF LITERATURE

Comprehensive reviews of the literature on the mosaic diseases of peas have been made by Pierce (*10*),[3] Zaumeyer and Wade (*16*), Stubbs (*11*), and Murphy and Pierce (*4*). Johnson and Jones (*1*) in 1937 described two mosaic diseases of pea in Washington, one an enation mosaic and the other a severe mosaic. The former is identical with the pea enation virus disease described by Osborn (*5*), Pierce (*10*), and Stubbs (*11*), which has been designated as pea virus 1. Severe mosaic, according to Johnson and Jones (*1*), appears to be similar to pea virus 3. Zaumeyer and Wade (*17*) recorded the varietal susceptibility of a number of pea varieties to pea streak virus 1, which is unlike any previously described virus of peas. Johnson and Lefebvre (*2*) reported a crotalaria mosaic that was infectious to a number of species of this host as well as to peas and other legumes. It is not known whether this virus is distinct from the viruses previously reported. Whipple and Walker (*13*) described two strains of the cucumber mosaic virus which infected pea and bean. Zaumeyer (*14*) compared pea streak virus 1 with three strains of the alfalfa mosaic virus, all of which infected pea. Wade and Zaumeyer (*12*) recorded varietal susceptibility and resistance of a number of pea varieties to a virus from alsike clover which is identical with the one described here as alsike clover mosaic virus 1. Osborn (*8*) recently reported on the incubation period of pea virus 1 in the aphid *Macrosiphum solanifolii* Ash. and later (*9*) reported strains of this virus.

[1] Received for publication January 11, 1940.

[2] The writer is indebted to L. L. Harter for suggestions and criticisms in the preparation of the manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 451.

Zaumeyer (*15*) compared two new viruses of the pea which resemble those reported by Whipple and Walker (*13*).

Pierce (*10*) arbitrarily classified a number of legume viruses which had been previously reported upon by him as well as by other investigators. His classification was based on the host from which the virus was collected, i. e., beans, peas, white clover, etc. Following this system of classification, the writer has designated the viruses herein discussed as pea mosaic viruses 4 and 5 and alsike clover mosaic viruses 1 and 2.

MATERIALS AND METHODS

SOURCES OF VIRUSES

Pea mosaic virus 4.—This virus was obtained from mosaic-infected peas grown in northeastern Colorado. The symptoms produced, which are described later, were in general mild on the several pea varieties tested (fig. 1, *A* and *B*).

Pea mosaic virus 5 (*pea stunt mosaic*).—Specimens from which this virus was isolated were collected from the Potlatch variety near Avon, Colo. This virus produced a decided stunting on the Dwarf Telephone variety (fig. 2, *A* and *F*).

Alsike clover mosaic virus 1.—This virus was obtained from a naturally infected alsike clover plant growing in northeastern Colorado. The symptoms produced on most pea varieties were mild and somewhat similar to those produced by pea mosaic virus 4 (fig. 3, *C* and *D*).

Alsike clover mosaic virus 2.[4]—This virus was secured from an alsike clover plant collected near Stonewall, Colo. It produced symptoms on alsike clover similar to those of alsike clover mosaic virus 1 but caused distinctly different symptoms on peas. Alsike clover mosaic virus 2 produced very marked symptoms on most varieties of peas (fig. 4, *A*,*B*, and *I*).

METHODS

The methods employed in these studies were similar to those described by other investigators who have worked with viruses of leguminous plants (*4*, *9*, *10*, *13*, *17*). The work was carried out entirely under greenhouse conditions at temperatures ranging from 18° to 25° C. Carborundum powder No. 38713 was dusted on the leaves before they were mechanically inoculated with the virus extracts.

The methods used in the studies of thermal inactivation and dilution were similar to those described by Johnson and Grant (*3*). For the aging tests, the juice of infected plants was stored in stoppered flasks and kept in a darkened chamber maintained at a constant temperature of 18° C. Juice was taken from the flasks at 24-hour intervals, and inoculations were made in the usual manner.

EXPERIMENTAL RESULTS

The viruses dealt with in this paper may be differentiated (1) by the symptoms they produce on several varieties of pea and bean; (2) by the susceptibility and resistance of certain varieties of pea,

[4] Since the preparation of this article, a new classification of the typical legume viruses has been published: WEISS, F. A KEY TO THE TYPICAL VIRUSES OF THE LEGUMINOUS CROPS. U. S. Bur. Plant Indus., Plant Dis. Rptr. 23: 352-360. 1939. [Mimeographed.]

In this classification, pea mosaic virus 4 has been changed to Pisum virus 3A, pea mosaic virus 5 to Pisum virus 5, alsike clover mosaic virus 1 to Trifolium virus 3, and alsike mosaic virus 2 to Trifolium virus 3A.

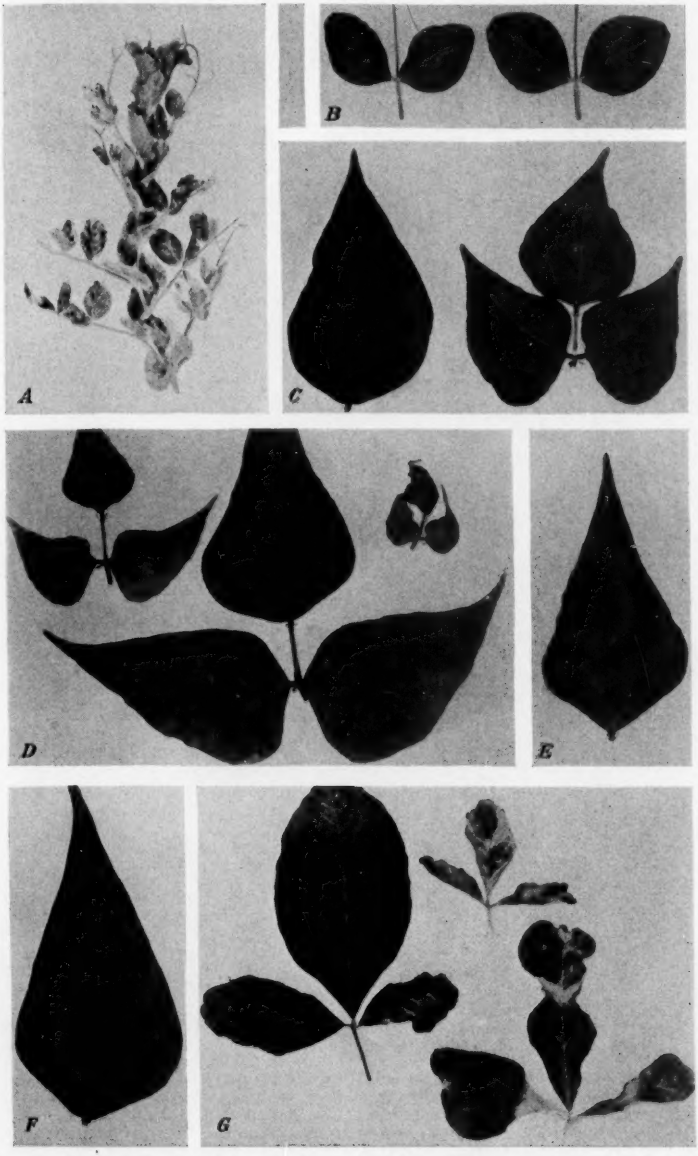

FIGURE 1.—Symptoms produced by pea mosaic virus 4 on several hosts: *A*, *B*, Leaf mottling on Green Giant pea; *C*, leaf mottling on Robust bean; *D*, leaf mottling on Stringless Green Refugee bean; *E*, *F*, halolike yellow spotting on U. S. No. 5 Refugee bean; *G*, leaf mottling and malformation on *Crotalaria spectabilis*.

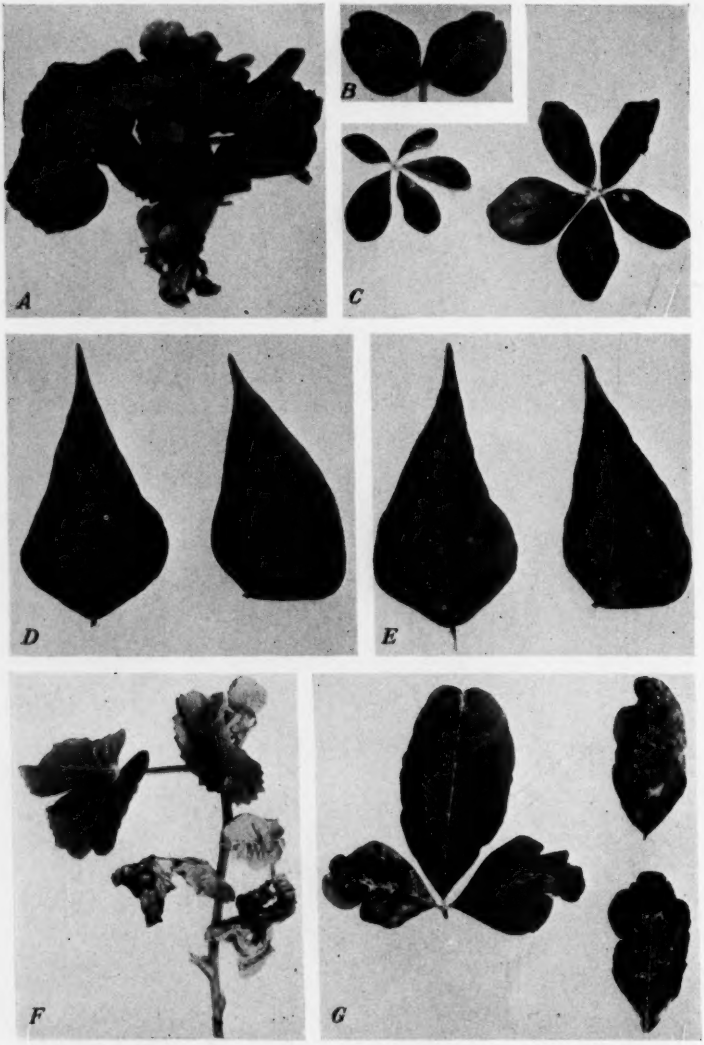

FIGURE 2.—Symptoms produced by pea mosaic virus 5 on several hosts: *A*, Stunting and terminal rosetting of Dwarf Telephone pea; *B*, leaf mottling on Green Giant pea; *C*, leaf malformation and necrosis on white lupine; *D*, mild leaf mottling on Idaho Refugee bean; *E*, leaf mottling on Stringless Green Refugee bean; *F*, leaf necrosis and terminal killing of Dwarf Telephone pea; *G*, leaf mottling and malformation on *Crotalaria spectabilis*.

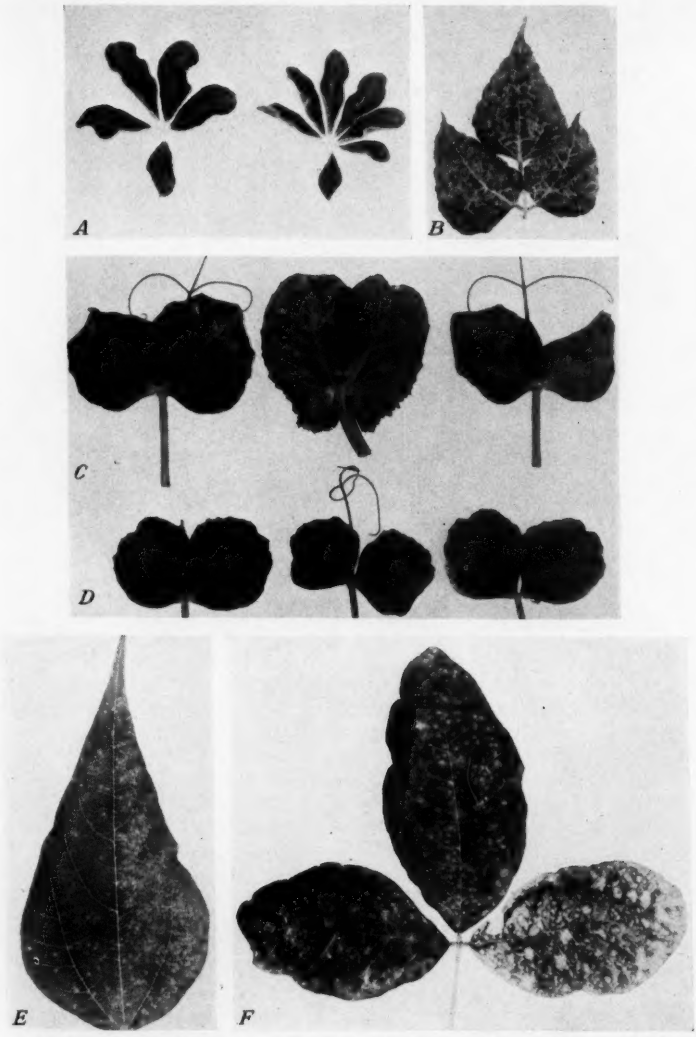

FIGURE 3.—Symptoms produced by alsike clover mosaic virus 1 on several hosts: *A*, Leaf mottling on white lupine; *B*, leaf mottling and puckering on Robust bean; *C*, *D*, leaf mottling on Dwarf Telephone and Green Giant pea, respectively; *E*, yellow leaf spotting on Stringless Green Refugee bean; *F*, yellow leaf spotting on *Crotalaria spectabilis*.

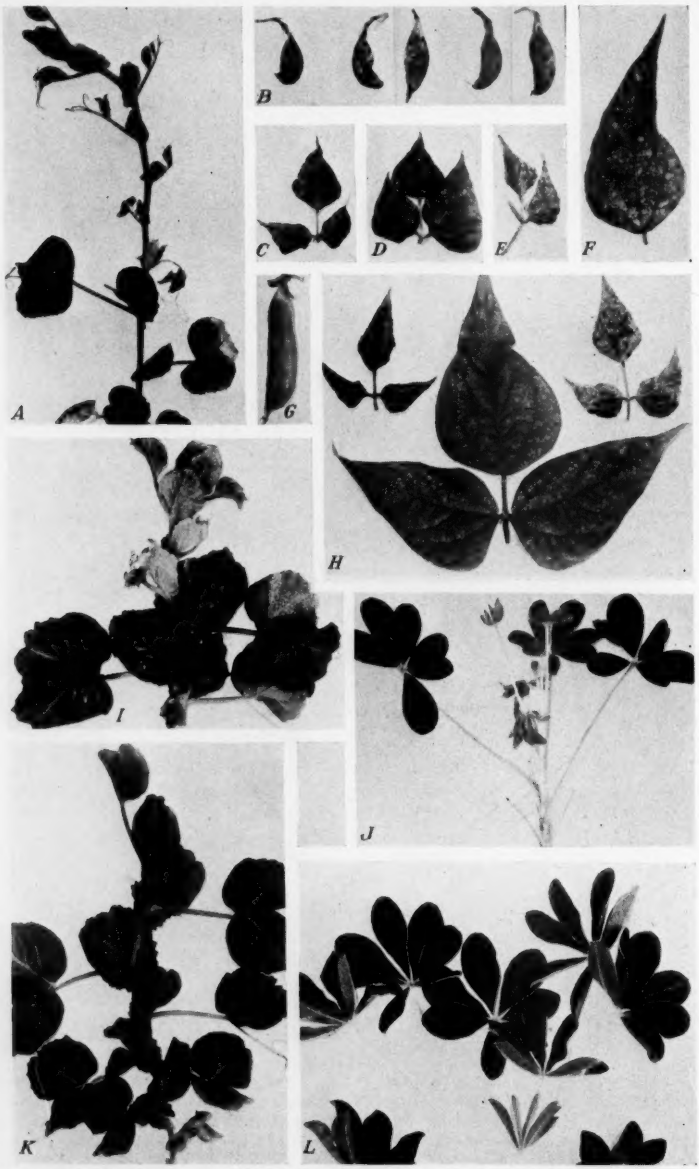

FIGURE 4.—Symptoms produced by alsike clover mosaic virus 2 on a number of hosts: *A*, Necrosis and killing of leaves of Green Giant pea; *B*, pod spotting on Alaska pea; *C*, *E*, *F*, leaf mottling and malformation on Corbett Refugee bean; *D*, leaf mottling on Robust bean; *G*, pod of healthy Alaska pea; *H*, trifoliate leaves of Idaho Refugee bean, showing yellow spotting and mottling; *I*, leaf spotting and necrosis on Dwarf Telephone pea; *J*, leaf malformation and death of white lupine; *K*, healthy Dwarf Telephone pea; *L*, healthy white lupine.

bean, and other legumes and hosts; and (3) by the properties of the viruses in vitro.

SYMPTOMS PRODUCED ON PEAS

Although symptomatology alone is not always sufficient to enable one to identify certain viruses on pea, a careful study of the symptoms produced on differential hosts is helpful. Some of the viruses here reported caused consistent symptoms on a number of pea varieties that can be relied on to help differentiate them. The descriptions of the symptoms produced by the several viruses are based on those that developed on Dwarf Telephone, Telephone, and Green Giant varieties, which were artificially inoculated in the greenhouse.

Pea Mosaic Virus 4

Pea mosaic virus 4 caused rather mild symptoms on most of the pea varieties tested (fig. 1, *A* and *B*). No leaf malformation or any appreciable stunting of the plant was produced. Infected plants showed a typical leaf mottling, the dark-green tissue being most frequently found adjacent to the veinlets. Small streaks or islands of yellowish tissue later appeared but never to the extent to which they were produced by pea mosaic viruses 1 and 3. Leaf necrosis was not observed. The symptoms somewhat resembled those reported by Stubbs (*11*) for pea viruses 2B and 2C.

Pea Mosaic Virus 5

Pea mosaic virus 5 (pea stunt mosaic) produced very similar symptoms on the Dwarf Telephone and Telephone varieties, but decidedly different symptoms on Green Giant.

On Dwarf Telephone and Telephone, stunting and dwarfing were characteristic features the plants reaching only about one-fourth natural size. Infected plants were only mildly mottled. The internodes were shortened, and the stem exhibited an intense purple discoloration followed by shrinking of the tissue. The vascular elements were discolored. About a week after inoculation the inoculated leaves were killed, and necrosis of the leaves about this region followed. Leaf necrosis was first evident at the base of the lamina, later becoming general over the entire leaflet. On Dwarf Telephone the growing point was often rosetted, the internodes were shortened, and the leaves were very compact, curled, and much smaller than normal (fig. 2, *A*). On both this variety and Telephone the terminal growth frequently became flaccid and died (fig. 2, *F*), and later the entire plant died. The leaves below the point of inoculation appeared normal.

On Green Giant only a slight stunting or leaf malformation was noted. Infected plants were decidedly mottled (fig. 2, *B*). In general, the lighter green tissue was found adjacent to the midrib and branch veins with darker islands adjacent to the veinlets. The light-green tissue later became yellow.

Alsike Clover Mosaic Virus 1

The symptoms produced by alsike clover mosaic virus 1 were much the same on Dwarf Telephone, Telephone, and Green Giant varieties. The leaves were typically mottled and bore symptoms resembling those

of pea virus 3 except that they were slightly less intense (fig. 3, *C* and *D*). They were, however, more striking than those produced by pea mosaic virus 4 (fig. 1, *A* and *B*). Leaves of diseased plants were lighter green than normal, with small to fairly large, irregularly shaped dark-green patches of tissue usually surrounding the small veinlets; larger areas of yellow frequently appeared later. The symptoms were quite similar to those produced by pea virus 2B of Stubbs (*11*). Infected plants were only slightly stunted and only minor foliage distortion or reduction in size of leaves and stipules was noted.

Alsike Clover Mosaic Virus 2

The symptoms produced by alsike clover mosaic virus 2 were distinctly different from and more severe than any thus far reported. They were quite similar on Dwarf Telephone, Telephone, and Green Giant, except that Dwarf Telephone was more stunted than the other varieties. Typical mottling such as was common with the three other viruses did not occur. The inoculated leaves usually died (fig. 4, *A*), as they did with the pea stunt virus. The leaves and stipules that arose at the first and sometimes the second node above the inoculated leaves, as well as those below the point of inoculation, remained normal, with the exception of an occasional leaflet exhibiting necrotic spots (fig. 4, *A* and *I*). Above this region the leaves were yellowish green and crinkled, and did not unfold so readily as normal ones. Infected leaflets were usually one-fourth to one-eighth normal size. The leaves arising from buds at the axils of the inoculated leaves, as well as offshoots arising from below the point of inoculation, showed the typical symptoms, which were later followed by the appearance of numerous small brown necrotic spots (fig. 4, *I*). Infected leaflets and stipules appeared water-soaked (fig. 4, *A* and *I*) and semitransparent. The green tissue was only found adjacent to the veins. These leaves finally died and fell off, leaving the bare stem and the malformed growing tip (fig. 4, *A*), which sometimes died later, leaving a few green normal-appearing leaves above the point of inoculation. The stems and petioles became very brittle and were readily broken. If pods were produced they were malformed and seriously spotted and pitted (fig. 4, *B*) and never reached maturity.

Resistance and Susceptibility of Pea Varieties

Twelve varieties of peas were inoculated with each of the viruses studied. For the sake of comparison, the varieties selected were in many cases identical with those employed by other students of legume viruses.

It is seen from table 1 that Horal, Little Marvel, Perfection, Surprise, and Wisconsin Early Sweet are resistant to the four viruses, whereas the other varieties are susceptible to all of them. The symptoms produced by the several viruses differed in intensity, depending upon the virus used and the variety tested. Pea mosaic virus 5 and alsike clover mosaic virus 2, in general, produced the most severe symptoms; pea mosaic virus 4 and alsike clover mosaic virus 1 produced milder symptoms. In practically all cases a high percentage of infection occurred; however, alsike clover mosaic virus 2 produced only 50 percent infection in Harrison Glory and 70 percent in Laxton Progress (table 1).

TABLE 1.—*Susceptibility and resistance of 12 pea varieties to pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2*

Variety	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Alaska	10	10b	12	12a	10	10c	10	8a
Dwarf Telephone	10	10b	11	11a	10	10b	10	10a
Green Admiral	10	10b	10	10b	13	13c	15	15a
Green Giant	10	9b	13	12b	10	10b	10	10a
Harrison Glory	11	11c	10	9b	10	9b	14	7a
Horal	12	0	10	0	12	0	10	0
Laxton Progress	18	18b	10	9a	10	10b	17	12a
Little Marvel	10	0	11	0	10	0	10	0
Perfection	10	0	10	0	10	0	10	0
Surprise	10	0	26	0	10	0	11	0
Telephone	10	10b	15	15a	10	10b	10	10a
Wisconsin Early Sweet	10	0	22	0	22	0	22	0

[1] Degree of infection denoted by letters: a=serious, b=moderate, c=mild.

In comparing differences in infectivity between the viruses reported herein and those of other workers, it is evident (see table 7) that the four viruses described by the writer differ from pea virus 1 (pea enation mosaic), pea virus 2 of Osborn (*6*), pea virus 3 (common pea mosaic), bean virus 2 (white sweetclover mosaic), white clover mosaic virus 1, pea streak virus 1, alfalfa mosaic viruses 1, 1A, and 1B, and the vein mosaic virus of red clover. Pea virus 1 is infectious to the Horal, Perfection, and Surprise varieties (see table 7), but these varieties were not infected by the viruses described herein (table 1). Pierce (*10*) reported pea virus 3 as infectious to all of the above-named varieties except Horal, whereas Murphy and Pierce (*4*), working with the same virus, reported these varieties to be resistant, except a strain of Surprise. Pierce (*10*) found Alaska and Surprise susceptible to bean virus 2, and all the differential pea varieties susceptible to white clover virus 1. Osborn (*7*) found Horal and Perfection susceptible to a vein mosaic virus of red clover. Zaumeyer (*14*) reported that all four differential varieties were susceptible to pea streak virus 1 and that only Horal was resistant to three strains of the alfalfa mosaic virus. Stubbs (*11*) reported pea viruses 2A, 2B, and 2C infectious to Surprise but not to Perfection. It is evident from these facts that the four viruses discussed in this paper differ from the previously reported legume viruses in infectivity to certain pea varieties.

SYMPTOMS PRODUCED ON BEANS

PEA MOSAIC VIRUS 4

The symptoms produced by pea mosaic virus 4 on Stringless Green Refugee bean were very similar in certain respects to those produced by bean virus 2 (white sweetclover mosaic virus). Yellow halolike spots (fig. 1, *E* and *F*) appeared on leaves above the leaves that were inoculated, but the spots were less distinct and more diffuse than those produced by the sweetclover mosaic virus. The infected leaves drooped at the pulvini; later, the uppermost leaves became quite

severely mottled, crinkled, and puckered; and the young trifoliate leaves were sometimes decidedly malformed (fig. 1, *D*). In these respects the symptoms were quite similar to those produced by the common bean mosaic. Infected plants showed considerable stunting and frequently died. On Robust bean, the yellow spotting was not so pronounced but the mottling was very characteristic (fig. 1, *C*). Infected plants sometimes showed considerable stunting. The symptoms produced on Idaho Refugee bean and U. S. No. 5 Refugee bean were identical. The symptoms were similar at first to those noted on Stringless Green Refugee but later were less intense.

Pea Mosaic Virus 5

Pea mosaic virus 5 (pea stunt mosaic), when inoculated on Stringless Green Refugee bean, produced symptoms that were very similar in many respects to those produced by white clover virus 1 on beans. The mottling was much milder than that produced by the mild pea mosaic virus but more intense than that produced by white clover virus 1 on beans. The older trifoliate leaves became slightly chlorotic and the mottling very blotchy with little malformation of the leaves (fig. 2, *D* and *E*). No appreciable stunting of the plants was noted. The symptoms were similar in all the susceptible varieties.

Alsike Clover Mosaic Virus 1

The symptoms produced by alsike clover mosaic virus 1 on beans resembled those produced by pea mosaic virus 5. Yellow halolike spots were noted on the trifoliate leaves (fig. 3, *E*), but they did not appear so quickly and were not so intense as those produced by pea mosaic virus 5. The yellow chlorotic blotching, which was characteristic on the trifoliate leaves, sometimes eventually caused considerable chlorosis and appeared somewhat rugose. Infected plants were only slightly stunted. On Robust bean no chlorosis was observed, but mottling, puckering, and slight blistering of the leaves were characteristic (fig. 3, *B*). On Corbett Refugee bean the symptoms were slightly milder than those on the other varieties except Robust.

Alsike Clover Mosaic Virus 2

Alsike mosaic virus 2 produced the most severe symptoms of any of the viruses. They were very similar to but more severe than those caused by pea mosaic virus 4. Yellow halolike spots appeared on the trifoliate leaves, and the leaflets later drooped at the pulvini. The youngest trifoliate leaves were mottled, puckered, and blistered, and smaller than normal (fig. 4, *C*, *D*, *E*, *F*, *H*). The internodes were shortened and the plants were extremely stunted. Later they became very chlorotic and many died. The symptoms were more severe and pronounced than those of the common bean mosaic. On U. S. No. 5 Refugee and on Robust the symptoms were not so severe as on the other susceptible varieties but in general they were similar.

RESISTANCE AND SUSCEPTIBILITY OF BEAN VARIETIES

The four viruses were inoculated on seven bean varieties, namely, Stringless Green Refugee, Corbett Refugee, Idaho Refugee, Wisconsin Refugee, U. S. No. 5 Refugee, Robust, and Great Northern U. I. No. 1. The results are shown in table 2.

Stringless Green Refugee, Idaho Refugee, U. S. No. 5 Refugee, and Robust were susceptible to pea mosaic virus 4, while Corbett Refugee, Wisconsin Refugee, and Great Northern U. I. No. 1 were resistant.

U. S. No. 5 Refugee was the only variety resistant to pea mosaic virus 5 (pea stunt mosaic). Great Northern U. I. No. 1 and Robust were very slightly susceptible to this virus, each having 1 infected plant out of 23 and 21 inoculated plants, respectively. It is probable that these were admixtures. The symptoms produced by pea mosaic virus 5 were milder than those produced by pea mosaic virus 4.

Great Northern U. I. No. 1 was resistant to alsike clover mosaic virus 1, while all of the other varieties were susceptible. Robust and Corbett Refugee exhibited the severest and the mildest symptoms, respectively.

All the varieties tested, except Great Northern U. I. No. 1, were susceptible to alsike clover mosaic virus 2. In general, the symptoms produced by this virus were more severe than those caused by any of the other viruses. U. S. No. 5 Refugee and Robust exhibited milder symptoms than any of the other varieties.

TABLE 2.—*Susceptibility and resistance of seven bean varieties to pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2*

Variety	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Stringless Green Refugee	21	21a	10	10c	22	17b	20	20a
Corbett Refugee	11	0	9	9c	12	9c	9	5a
Idaho Refugee	11	5b	10	10c	11	8b	10	6a
Wisconsin Refugee	11	0	10	10c	10	6b	11	7a
U. S. No. 5 Refugee	12	12b	12	0	11	8b	12	4b
Great Northern U. I. No. 1	9	0	23	1c	12	0	11	0
Robust	11	8b	21	1c	12	12a	13	4b

[1] Degree of infection denoted by letters: a=serious, b=moderate, c=mild.

These results clearly show that these four viruses are unlike most of those reported earlier on legumes with respect to the susceptibility and resistance of certain bean varieties (see table 7). Pea virus 1, pea virus 3, pea streak virus 1, and vein mosaic virus of red clover are not infectious to bean, and in this regard differ from the four viruses discussed in this paper. Pea viruses 2A, 2B, and 2C of Stubbs (*11*) did not infect Stringless Green Refugee and Corbett Refugee, the only two varieties tested. Bean virus 2 and white clover virus 1, as shown by Pierce (*10*), infected all of the varieties listed in table 2 with the exception of U. S. No. 5 Refugee, which was not tested. In this respect these two viruses are similar to pea mosaic virus 5 (pea stunt mosaic), which infected all of the varieties tested except U. S. No. 5 Refugee. The percentage of infection with this virus (table 2) for Great Northern U. I. No. 1 and Robust was lower than that reported by Pierce (*10*) with white clover virus 1 and bean virus 2 for these same varieties. Furthermore, the symptoms produced by bean virus 2 were more severe than those produced by pea

mosaic virus 5. Those produced by white clover virus 1 and by pea mosaic virus 5 were of about equal intensity.

Pea virus 2 of Osborn (*6*) was found to infect Stringless Green Refugee, Corbett Refugee, and Robust, but not Great Northern U. I. No. 1. These varieties reacted similarly when inoculated with alsike clover mosaic viruses 1 and 2 (table 2). The symptoms produced by pea mosaic virus 2 on Stringless Green Refugee were not greatly unlike those manifested by alsike clover mosaic viruses 1 and 2 on this variety. These viruses produced a distinct mottling on the trifoliate leaves which was slightly more severe in the case of the alsike mosaic viruses than in that of pea virus 2. Osborn (*6*) reports a mild mottling on Robust, while alsike clover mosaic virus 1 produced a severe mottling and alsike clover mosaic virus 2 a mottling of moderate intensity. The alfalfa mosaic viruses 1, 1A, and 1B, according to Zaumeyer (*14*), produced local lesions on all of the bean varieties tested, whereas the four viruses described in this paper produced systemic infection.

The severe pea mosaic (see table 7) of Johnson and Jones (*1*) was reported to have infected all of the bean varieties except U. S. No. 5 Refugee, Wisconsin Refugee, and Idaho Refugee, which were not tested.

OTHER HOSTS

A relatively small number of hosts were inoculated with the four viruses to determine, if possible, differences in susceptibility and resistance. The results are shown in table 3. It is clearly evident that of these the susceptible hosts were restricted to the Leguminosae. It is also shown that, with few exceptions, hosts that were susceptible to one virus were susceptible to all four. *Crotalaria spectabilis* was susceptible to all the viruses except alsike clover mosaic virus 1. Pea mosaic virus 5 was the only virus infectious to *C. retusa*, while *C. striata* was susceptible to all the viruses. Only one plant of *Trifolium pratense* became infected with pea mosaic virus 4, and none with the other viruses. *Lupinus albus*, *L. angustifolius*, *Medicago sativa*, *Melilotus alba*, *Trifolium incarnatum*, and *Vicia faba* were susceptible to all of the viruses tested.

These data also show that the four viruses were dissimilar to the pea mosaic viruses reported by other investigators (see table 7). Pea virus 1, pea viruses 2A, 2B, and 2C, and pea virus 3 were not infectious to white sweetclover, differing in this respect from the four viruses discussed in this paper. Bean virus 2, pea virus 2, pea virus 3, white clover virus 1, the alfalfa mosaic viruses 1, 1A, and 1B, pea streak virus 1, vein mosaic of red clover, and severe pea mosaic were all infectious to red clover. Pea mosaic virus 4 was the only one reported here which infected this host. Pea mosaic virus 4 differed from bean virus 2, the three alfalfa mosaic viruses, and pea streak virus 1 in not being infectious to soybean. It was unlike white clover virus 1 and severe mosaic in that it was not infectious to white clover. The symptoms produced on red clover by the vein mosaic virus of red clover, according to Osborn (*7*), differed widely from those produced by pea mosaic virus 4.

TABLE 3.—*Susceptibility and resistance of various legumes and other plants to pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2*

Host	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Crotalaria spectabilis Roth (No. 18096)	4	2a	4	2b	4	0	4	2a
C. retusa L. (No. 65331)	5	0	5	4b	5	0	5	0
C. striata DC. (No. 19322)	5	5b	5	5b	5	5b	5	5a
Lupinus albus L. (white lupine)	10	10a	10	10a	10	10b	10	10b
L. angustifolius L. (blue lupine)	10	9b	10	8b	10	8a	8	7a
Medicago sativa L. (alfalfa)	5	2c	5	3c	.....	.....	5	1c
Melilotus alba Desr. (white sweetclover)	9	2b	9	6b	8	2b	9	4c
Phaseolus aureus Roxb. (mung bean)	10	0	10	0	10	0	10	0
P. lunatus L. (sieva bean, Henderson Bush variety)	11	0	10	0	10	0	10	0
Soja max (L.) Piper (soybean, Virginia variety)	10	0	10	0	10	0	10	0
S. max (L.) Piper (soybean, Manchu variety)	10	0	10	0	10	0	10	0
Trifolium incarnatum L. (crimson clover)	5	5b	5	5a	5	5a	5	5a
T. pratense L. (red clover)	5	1b	5	0	5	0	5	0
T. repens L. (white clover)	9	0	9	0	9	0	9	0
Vicia faba L. (broadbean)	10	10b	10	9b	10	10b	10	10c
V. sativa L. (spring vetch)	10	0	10	0	10	0	10	0
Datura stramonium L. (jimsonweed)	10	0	10	0	10	0	10	0
Nicotiana glauca Graham	10	0	10	0	10	0	10	0
N. glutinosa L.	10	0	10	0	10	0	10	0
N. tabacum L. (Turkish tobacco)	10	0	10	0	10	0	10	0
Petunia hybrida Vilm. (petunia)	10	0	10	0	10	0	10	0

[1] Degree of infection denoted by letters: a=serious, b=moderate, c=mild.

PROPERTIES OF THE VIRUSES

Certain properties of the four viruses were determined in order to detect differences among them as well as to distinguish them from other known legume viruses. The thermal inactivation points, resistance to aging in vitro, and tolerance to dilution were determined. These studies were made with peas as the test host; however, the inoculum was not in every case prepared from infected pea plants.

Thermal inactivation point.—When infectious plant juice was heated for 10 minutes (table 4), the thermal inactivation points for the different viruses were as follows: Pea mosaic virus 4, 62° to 65° C.; pea mosaic virus 5 and alsike clover mosaic virus 1, 60° to 62°; and alsike clover mosaic virus 2, 54° to 58°.

Tolerance to dilution.—In regard to tolerance to dilution, pea mosaic virus 4, pea mosaic virus 5, and alsike clover mosaic virus 2 were still somewhat infectious at a dilution of 1 to 8,000 (table 5). Alsike clover mosaic virus 1 was infectious at 1 to 6,000, but not at 1 to 8,000.

Resistance to aging in vitro.—Pea mosaic virus 4 and alsike clover mosaic viruses 1 and 2 were no longer infectious after aging from 1 to 2 days (table 6). Pea mosaic virus 5 lost its infectivity in less than 1 day.

In general, the thermal inactivation points of the four viruses showed no outstanding differences from those of previously described legume viruses (see table 7). Pea mosaic viruses 4 and 5 were inac-

tivated at slightly higher temperatures than were white clover virus 1 and bean virus 2 (table 7), but the differences were not significant. Alsike clover mosaic virus 2 was killed at a somewhat lower temperature than the other viruses.

TABLE 4.—*Thermal inactivation points of pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2, as determined by systemic infection of peas*

Temperature [1] (°C.)	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5 (pea stunt mosaic)		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Not heated	20	18	23	15	20	20	25	25
54	27	13	30	7	30	22	29	5
58							25	0
60	29	6	30	2	27	2	30	0
62	27	1	30	0	30	0	30	0
65	25	0	25	0	30	0	30	0

[1] Virus was heated for 10 minutes at temperature indicated.

TABLE 5.—*Tolerance to dilution of pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2, as determined by systemic infection of peas*

Dilution	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5 (pea stunt mosaic)		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
None	24	23	20	17	22	22	20	13
1:2,000	12	9	15	2	16	5	9	7
1:4,000	12	8	29	4	16	5	12	5
1:6,000	33	9	30	1	31	1	32	4
1:8,000	30	2	30	1	30	0	30	1

TABLE 6.—*Resistance to aging in vitro of pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), and alsike clover mosaic viruses 1 and 2, as determined by systemic infection of peas*

Time aged at 20° C. (days)	Reaction to—							
	Pea mosaic virus 4		Pea mosaic virus 5 (pea stunt mosaic)		Alsike clover mosaic virus 1		Alsike clover mosaic virus 2	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
0	20	18	20	20	20	18	10	10
1	23	17	20	0	26	5	26	5
2	20	0	20	0	22	0	26	0
3	20	0	20	0	22	0	20	0
4	12	0	10	0	12	0	12	0
5	12	0	10	0	12	0	12	0

Regarding longevity in vitro, pea mosaic virus 5 lost its infectiousness in less than 24 hours, which was a shorter time than was required by any of the other viruses except pea mosaic viruses 2A, 2B, and 2C, which were no longer infectious after ½ to 1 day (table 7). Pea mosaic virus 4 and alsike clover mosaic viruses 1 and 2 remained infectious from 1 to 2 days, resembling in this respect bean virus 2 and pea streak virus 1. Pea virus 2, severe pea mosaic, white clover virus 1, and the alfalfa mosaic viruses remained viable longer than any of the other viruses listed.

In general, the four viruses reported herein were inactivated at higher dilutions than other legume viruses with the exception of pea streak virus 1, which lost its activity at about the same dilution, and pea virus 2, which was infectious at 1 to 100,000 dilution. Johnson and Jones (*1*) reported that severe pea mosaic was also infectious at a dilution of 1 to 100,000.

DISCUSSION

The data show that three of the viruses here described are different from previously described viruses that are infectious to pea. The fourth, alsike clover mosaic virus 1, is identical with a previously described mosaic virus from alsike clover (*12*). With the possible exception of alsike clover mosaic virus 2, these viruses produced symptoms on peas not greatly unlike those caused by certain other leguminous viruses. On particular varieties, such as Dwarf Telephone, pea mosaic virus 5 produced symptoms different from most of the other known viruses, except the alfalfa viruses which produced somewhat similar symptoms on this variety. It has been pointed out repeatedly that symptoms alone are not in general a dependable criterion for the differentiation of viruses but are of considerable help when coupled with other features.

Wherever possible the same varieties of peas, beans, and other hosts that were used by other investigators were employed in this study, and this made it possible to compare better the four viruses discussed in this paper with those previously described. In table 7 are listed a few of the differentiating features of most of the viruses that have been shown to be infectious to pea. All of the viruses were not tested on each host. The data on viruses other than the four discussed here were compiled from previous publications of the writer as well as from publications of other authors.

The four viruses described herein differed from most of the other legume viruses in not being infectious to Horal, Perfection, and Surprise pea varieties. Pea mosaic virus 2 was tested only on the Perfection variety, which was resistant. Pea mosaic virus 4 differed from the viruses that were infectious to bean in that it did not infect Great Northern U. I. No. 1 and Corbett Refugee (table 7). Pea mosaic virus 5 and alsike mosaic viruses 1 and 2 were not infectious to red clover, which distinguished them from the other legume viruses except pea virus 1 and pea viruses 2A, 2B, and 2C. They differed from these last-named viruses in that they were infectious to a number of bean varieties that were not infected by these viruses (table 7).

TABLE 7.—*List of viruses affecting pea, showing differentiating features for the purpose of identification and classification* [1]

Virus	Reaction of [2]—															Characteristics of virus			
	Differential pea varieties				Differential bean varieties							Other hosts				Physical properties		Dilution	Other characteristics
	Alaska	Horal	Perfection	Surprise	Stringless Green Refugee	Robust	Great Northern U. I. No. 1	Corbett Refugee	Wisconsin Refugee	Idaho Refugee	U. S. No. 5 Refugee	Red clover	White sweetclover	White clover	Soybean	Thermal inactivation point	Longevity in vitro		
																°C.	Days		
Pea virus 1 (enation mosaic) (*9, 10*).	+	+	+	+	−	−	−	−	−	−		−	−	−	+	64–66	4–5	1:10,000	Production of leaf enation on peas and crimson clover.
Pea virus 2 (*6, 10*)			−		+	+	−	+				+	+	−		62–64	4–5	1:100,000	Brilliant mottling of leaves of peas and crimson clover.
Pea viruses 2A, 2B, 2C (*11*)	+		−	+	−			−				−	−	−	−		1/2–1	1:1,000 to 1:2,000	
Pea virus 3 (common pea mosaic) (*4, 10*).	+	−	−	+	−	−	−	−				+	−	−	−	62–64	2–3		
Severe pea mosaic (*1*)			+		+	+	+	+				+		+		60–70	15	1:100,000	
Bean virus 2 (white sweetclover mosaic) (*10*).	+	−	−	+	+	+	+	+	+	+		+	+	−	+	58–60	1–2	1:800 to 1:1,000	Considerable yellowing of leaves of beans and peas.
White clover virus 1 (*10, 16*)	+	+	+	+	+	+	+	+	+	+		+	+	+	−	58	6–7	1:2,000 to 1:3,000	Very mild mottling on leaves of peas and beans; necrosis of peas.
Alfalfa mosaic viruses 1, 1A, and 1B (*14*).	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	65–75	4–5	1:2,000 to 1:4,000	Production of local lesions on bean leaves.
Pea streak virus 1 (*14, 17*)	+	+	+	+	−	−	−	−	−	−	−	+	+	+	+	62–65	1–2	1:5,000 to 1:8,000	Intense streaking of stems of peas and terminal necrosis.
Vein mosaic virus of red clover (*7*).		+	+		−	−	−	−				+	+	+		58–60	2–3		Clearing of veins of peas, red clover, and *Vicia faba*.
Pea mosaic virus 4	+	−	−	−	+	+	−	−	−	+	+	+	+	−	−	62–65	1–2	1:8,000 to 1:10,000	
Pea mosaic virus 5 (pea stunt mosaic).	+	−	−	−	+	+	+	+	+	+	−	−	+	−	−	62–65	0–1	1:8,000 to 1:10,000	Severe stunting of Dwarf Telephone and Telephone pea varieties.
Alsike clover mosaic virus 1	+	−	−	−	+	+	−	+	+	+	+	−	+	−	−	60–62	1–2	1:6,000 to 1:8,000	
Alsike clover mosaic virus 2	+	−	−	−	+	+	−	+	+	+	+	−	+	−	−	54–58	1–2	1:8,000 to 1:10,000	Extreme leaf necrosis and drop of leaves of peas.

[1] For reference to the reclassification of the legume viruses, see footnote 4.
[2] Plus sign (+) represents infection; minus sign (−) represents no infection.

Zaumeyer and Wade (*16*) earlier reported an alsike clover mosaic virus which they named alsike clover mosaic virus 1. The symptoms that it produced on alsike clover, peas, and beans were very similar to those produced by alsike clover mosaic virus 1 herein reported. The only difference between the two viruses is that the earlier reported virus was slightly infectious to Perfection and Little Marvel peas, to which the virus of this study was not infectious. These differences might have been due to different strains or to admixtures of the varieties. In other respects the two appeared almost identical and hence are considered as the same virus. The alsike clover virus reported by Wade and Zaumeyer (*12*) is also identical with alsike clover mosaic virus 1.

The chief characteristic that differentiates pea virus 1 from the other viruses that are infectious to pea is the production of leaf enations on both peas and crimson clover, a symptom produced by none of the other legume viruses thus far described. Stubbs (*11*) reported that pea virus 1 produced severe top necrosis on seedlings of Dwarf Telephone pea. This is similar to the symptom produced by pea mosaic virus 5 on this variety.

Pea virus 2, described by Osborn (*6*), although not tested on all the hosts listed in table 7, differs from several of the viruses in its inability to infect Perfection pea. It differs from other viruses that infect this variety in the reaction to it of certain bean varieties. It most closely resembles alsike clover mosaic viruses 1 and 2, but these two viruses are not infectious to red clover which is susceptible to pea virus 2 (table 7). It also differs from these viruses in its ability to withstand aging longer in vitro. The symptoms produced by pea virus 2 on pea are markedly different from those produced by alsike clover mosaic virus 2 on this host.

Stubbs' pea viruses 2A, 2B, and 2C (*11*) closely resembled pea mosaic virus 3 in the symptoms and reactions that they caused to pea varieties (table 7). The principal points of difference were in the immunity of Perfection pea and the susceptibility of red clover to pea mosaic virus 3. Although red clover was not readily infected artificially with certain other viruses, it is probable that future studies may prove this host susceptible in some degree to these viruses. It is believed that these three viruses are possibly strains of pea mosaic virus 3.

Pea mosaic virus 3 was similar to bean mosaic virus 2 in its infectiousness to certain pea varieties but differed in not being infectious to bean (table 7).

Severe mosaic of peas, described by Johnson and Jones (*1*), was tested only on one of the four pea varieties listed in table 7, namely, Perfection, to which it was found to be infectious. It resembled white clover virus 1 in its host reaction, but the symptoms it produced on peas and beans were decidedly different from those produced by white clover virus 1. In certain of its properties this virus differed greatly from most of the legume viruses that have been reported as infectious to pea. Its longevity in vitro was 15 days, and it was still infectious at a dilution of 1 to 100,000 (table 7). None of the other viruses was viable beyond a dilution of 1 to 8,000, except pea virus 2. Johnson and Jones (*1*) considered severe pea mosaic similar to pea virus 3 and to white sweetclover mosaic. Since it does not infect beans it does not appear closely related to pea virus 3.

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In host range, bean mosaic virus 2 most closely resembled the alfalfa mosaic viruses but differed markedly in the symptoms it produced on peas and beans. Their physical properties were also distinctly different (table 7). Perfection pea was immune to bean mosaic virus 2 but susceptible to the alfalfa viruses (table 7).

White clover mosaic virus 1 differed from the alfalfa mosaic viruses, which it most closely resembled, in the production of systemic infection in beans. The alfalfa mosaic viruses produced only local lesions on this host. Likewise, white clover mosaic virus 1 was infectious to Horal pea, while the alfalfa mosaic viruses were not. The thermal inactivation point for the alfalfa mosaic viruses was considerably higher than that for white clover virus 1.

Pea streak virus 1 differed from all of the other viruses in the fact that it produced a decided streaking of the stems of peas. The alfalfa mosaic viruses may also cause some streaking, but they differ from pea streak virus 1 in their ability to infect beans.

The vein mosaic of red clover reported by Osborn (*7*) most closely resembled pea mosaic virus 1, except that the latter was not infectious to red clover, white sweetclover, and white clover (table 7). Moreover, the symptoms produced by these two viruses on peas were unlike.

From the similarity in reaction of the several pea and bean varieties and other hosts to the four viruses discussed herein, it would appear that several of them are somewhat closely related. Likewise the physical properties of several of these viruses are quite similar. The thermal inactivation points, longevity in vitro, and dilution points for the four viruses dealt with in this paper were not sufficiently different to afford a basis for separating them. Table 7 shows that the thermal inactivation points of all of the legume viruses are approximately the same, and it is doubtful whether any of them could be separated by this means. Regarding longevity in vitro, the only viruses that were strikingly different from the four discussed here were pea virus 2, severe pea mosaic, white clover virus 1, and the alfalfa viruses. The four viruses discussed herein were infectious at higher dilutions than the other legume viruses except pea streak virus 1, which was still infectious at approximately the same dilution, and severe pea mosaic, which was reported by Johnson and Jones (*1*) as infectious at a dilution of 1 to 100,000.

Probably other new legume viruses will be described in the future. It is believed that such studies could be simplified by the employment by the different workers of standardized methods of technique and the use of the same differential varieties of peas, beans, and other hosts.

SUMMARY

Three new mosaic diseases infectious to pea, together with an earlier described mosaic virus from alsike clover, likewise infectious to pea, are described herein and identified.

The viruses studied were pea mosaic virus 4, pea mosaic virus 5 (pea stunt mosaic), alsike clover mosaic virus 1, and alsike clover mosaic virus 2. They were differentiated from one another and from the previously described legume viruses (1) by the symptoms they produced on peas and beans, (2) by the susceptibility and resistance of several varieties of pea, bean, and other legumes and of other hosts, and (3) by certain physical properties.

Pea mosaic virus 4 and alsike clover mosaic virus 1 produced comparatively mild symptoms on pea. Pea mosaic virus 5 produced considerable stunting of Dwarf Telephone and Tall Telephone pea varieties. Alsike clover mosaic virus 2 caused the most severe symptoms on peas, producing necrosis and leaf drop.

Twelve varieties of peas were inoculated with the four viruses. All were susceptible except Horal, Little Marvel, Perfection, Surprise, and Wisconsin Early Sweet.

The four viruses were infectious on certain bean varieties, but there were considerable varietal differences. Stringless Green Refugee was susceptible to the four viruses. Great Northern U. I. No. 1 was resistant to all of the viruses except pea mosaic virus 5. The viruses likewise produced different symptoms on the several varieties.

Pea mosaic virus 4 was the only one of the four viruses to infect red clover. The susceptible hosts were restricted to the Leguminosae. With few exceptions, hosts that were susceptible to one virus were susceptible to all.

Slight differences were noted between the physical properties of the four viruses affecting pea and those of viruses previously described. These differences were not sufficiently great to serve as the basis for definite identification and separation.

Certain important characteristic features of most of the viruses affecting pea are tabulated (table 7). The differences in most cases are sufficient for the purposes of differentiation and classification.

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PARTIAL SELF-INCOMPATIBILITY AND THE COLLAPSE OF FERTILE OVULES AS FACTORS AFFECTING SEED FORMATION IN ALFALFA[1]

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INTRODUCTION

Various investigators have confirmed the fact, since Piper et al. (*10*)[3] first reported it, that alfalfa (*Medicago sativa* L.) forms seed more freely after cross-pollination than after self-pollination. The authors (*4*) in a preliminary account, have shown that two probably distinct phenomena are involved. In the first place, it is found that alfalfa is partly self-incompatible. That is to say, male gametophytes are less effective in accomplishing fertilization in the plant from which they arise than in unrelated individuals. Moreover, there is a difference in survival of ovules containing inbred and hybrid embryos and endosperms, the former class being much more prone to collapse during development than the latter. The observations upon which these findings rest are presented in detail in the present paper and are considered in relation to the problem of seed setting in alfalfa.

Self-incompatibility is usually measured in terms of fruit and seed production. This may not be adequate in alfalfa in view of the possibility that ovule abortion, which also has a major effect on fruit and seed production, may be unrelated to self-incompatibility and may vary independently of it. The two phenomena have been separated in the present study by a histological study of the pistil following the two types of matings.

MATERIALS AND METHODS

Seven plants derived by self-pollination from six unrelated individuals selected from commercial varieties of alfalfa were used in the experiment. They were brought into flower in pots in a greenhouse at 70° to 80° F. in April, supplementary illumination being used to give a photoperiod similar to that in midsummer. The matings were made at the height of the flowering period. Newly opened flowers were castrated by clipping off the standard, forcing the sexual column out of the keel, and removing the pollen by suction. Some of the flowers were then selfed, using pollen from other flowers on the same respective plants. The remaining castrated flowers were cross-pollinated, the pollen being taken in each case from a different and unrelated plant within the group. The matings were so arranged that each of the plants, in addition to being selfed, served as a pistillate and as a staminate parent in the crosses. Intimate contact between the stig-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 472.

matic secretion and the pollen was assured by applying the pollen with toothpicks tipped with a strip of fine emergy cloth.

The pistils were collected at 30, 48, 72, 96, 120, and 144 hours after pollination, fixed in Karpechenko's modification of Navashin's fluid, and embedded in paraffin. After the material had been sectioned and stained with Delafield's haematoxylin, the following data were taken on each pistil, regard being given to the serial order of the ovules from apex to the base of the ovary: Position of the most advanced pollen tubes, fertility of the ovules, stage of development of the proembryo or embryo, and fertile ovules collapsing. The technique employed did not permit of following pollen-tube growth in the style. Within the cavity of the ovary, however, the pollen tubes grow along the surface of the ventral suture, and, at this stage, the tips of the longest tubes can be distinguished clearly, although the distribution of the less advanced tubes cannot be determined accurately. About nine pistils per plant were taken at each collection in the self-pollinated series and about six pistils per plant in the crossed group.

SEED PRODUCTION FOLLOWING SELF- AND CROSS-POLLINATION

The comparative fertility on self- and cross-pollination of the seven plants used, measured in terms of mature seeds produced, is shown in table 1. The data are based on a separate series of matings made during the same period as the test pollinations. The flowers in the selfed series, however, were not castrated, and the pollen used in the crosses was derived mostly from unrelated plants not otherwise included in these experiments. It is not believed that either of these circumstances should influence the results significantly.

TABLE 1.—*Fertility measured in terms of mature seeds produced, following self- and cross-pollination*

Plant (pistillate parent)	Self-pollination				Cross-pollination			
	Flowers	Pods	Seeds	Seeds per flower	Flowers	Pods	Seeds	Seeds per flower
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
A [1]	107	66	164	1.53				
B	74	26	43	.58	30	27	81	2.70
C	51	9	8	.16	24	22	133	5.54
D	105	45	78	.74	54	45	216	4.00
E	67	49	118	1.76	24	19	120	5.00
F	35	15	33	.94	76	69	450	5.92
G	43	15	27	.63	80	59	310	3.87
Total or average	375	159	307	.80	288	241	1,310	4.50

[1] Omitted from total.

Omitting plant A, for which the data are incomplete, it may be seen from table 1 that, on an average, about five and one-half times as many mature seeds developed per flower pollinated after crossing as after selfing. The values vary rather widely from plant to plant, particularly in the selfed series, but in the case of each individual a large increase is shown on crossing. This result is in conformity with the findings of Piper et al. (*10*), Frandsen (*8*), and Carlson (*5*).

The fertility differential between the crossed and selfed series is much wider when measured in terms of seeds formed per flower pollinated than in terms of pods set per flower pollinated. This follows

from the fact that the pods formed after selfing contained only 1.93 seeds each, on an average, whereas those in the cross-pollinated series contained 5.44 seeds.

OVULE FERTILITY

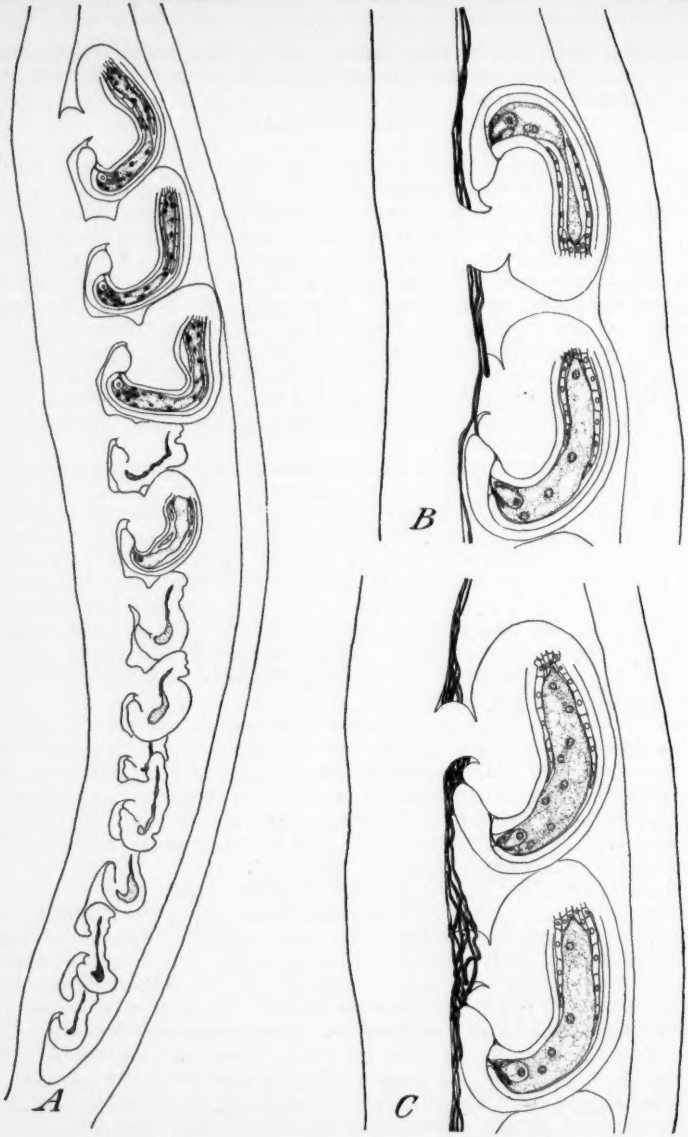
A primary cause of the disparity in the number of seeds that develop after self- and cross-pollination is found in the difference in the proportion of ovules that become fertile following the two types of matings. The ovary of alfalfa usually contains 10 to 12 ovules arranged alternately along the ventral suture in serial order. The ovules are comparatively uniform in size and, almost without exception, develop normal appearing embryo sacs. A necessary condition for the development of the seed is that fertilization of the egg within the ovule occur. A histological study of the ovary during the period immediately following completion of pollen-tube growth enables one to measure directly the frequency with which fertilization takes place. Failure of fertilization can thus be separated from other factors subsequently affecting fertility.

Just prior to fertilization the mature ovule is in a quiescent state. There is little, if any, evidence of nuclear or cell division in any of its parts. The pollen tube enters the embryo sac between the apices of the synergids and the egg. In the process of fertilization one male gamete nucleus fuses with the egg nucleus and the other unites with the fusing polar nuclei. Coincindental with fertilization active nuclear and cell division is stimulated in the integuments and funiculus and there is a considerable increase in the size of the ovule within a short period of time. The primary endosperm nucleus has oftentimes divided twice and there are four endosperm nuclei present at the time of the division of the zygote.

The fertilized ovules at 48 hours after pollination may be distinguished from those that have not been fertilized by the increase in size of the ovule, the development of the multinucleate endosperm, and the presence of a proembryo. If fertilization does not occur there is no further development of the ovule, the embryo sac collapses and ultimately the entire ovule, so that only a vestige of it remains at the later stages of the developing pod (pl. 1, *A*).

At 72 hours after pollination pollen tubes, which may be seen at certain earlier stages, can no longer be found within the cavity of the ovary. Fertilization, therefore, has ended, and a legitimate estimate of the frequency with which the process occurs after a given mating may be made. The results of an examination of ovaries collected at 72, 96, 120, and 144 hours after pollination are presented in table 2 and depicted graphically in figure 1. The samples collected at the different times provide the basis for four independent estimates of the percentage of fertile ovules in each plant in each of the two series.

It is evident from table 2 that a much larger proportion of the ovules become fertile after cross-pollination than after self-pollination. The respective mean values are 66.2 and 14.6 percent. Furthermore, the values for the two series are separated by a wide gap. The most fertile plant in the selfed series, B, shows 21.5 percent of the ovules containing a fertilized egg. The least fertile plant on crossing, which likewise proves to be B, has over twice as many fertile ovules. A statistical examination of the data brings to light additional relations not otherwise clearly apparent.



A, Median longitudinal section of an ovary 72 hours after self-pollination. The three apical ovules and the fifth ovule have been fertilized, the latter being in a stage of collapse. The remaining ovules have not been fertilized and are in stages of degeneration. × 425. *B*, Median longitudinal section of a portion of an ovary showing the two apical ovules 30 hours after self-pollination. The second ovule has been fertilized. The pollen tubes are few in number. × 850. *C*, Median longitudinal section of a portion of an ovary showing the two apical ovules 30 hours after cross-pollination. Both ovules have been fertilized. Pollen tubes are abundant, especially in the regions of the micropyles. × 850.

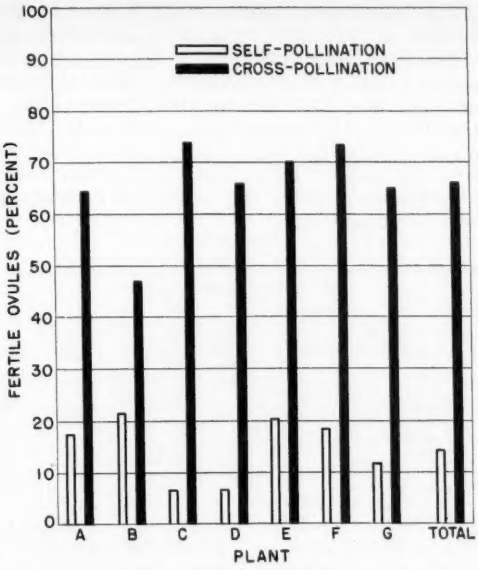


FIGURE 1.—Frequency of fertilization in individual plants following self-pollination and cross-pollination. Data based on pistils collected at 72, 96, 120, and 144 hours after pollination.

TABLE 2.—*Comparative fertility of ovules in individual plants following self- and cross-pollinations; data based on collections at 72, 96, 120, and 144 hours after pollination*

Plants selfed or crossed	72 hours			96 hours			120 hours			144 hours			Total		
	Total	Fertile		Total	Fertile		Total	Fertile		Total	Fertile		Total	Fertile	
Selfed:	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*
A	58	10	17.2	82	21	25.6	58	4	6.9	12	2	16.7	210	37	17.6
B	44	14	31.8	58	10	17.2	26	5	19.2	44	8	18.2	172	37	21.5
C	76	10	13.2	79	4	5.1	82	5	6.1	60	1	1.7	297	20	6.7
D	82	5	6.1	73	2	2.7	72	6	8.3	20	4	20.0	247	17	6.9
E	65	9	13.8	43	15	34.9	47	6	12.8	37	9	24.3	192	39	20.3
F	136	29	21.3	167	28	16.8	153	25	16.3	130	27	20.8	586	109	18.6
G	127	10	7.9	137	12	8.8	125	23	18.4	54	10	18.5	443	55	12.4
Total	588	87	.....	639	92	.....	563	74	.....	357	61	.....	2,147	314	14.6
Crossed:															
A×B	58	34	58.6	81	53	65.4	77	52	67.5	73	48	65.8	289	187	64.7
B×C	58	25	43.1	61	26	42.6	60	30	50.0	54	29	53.7	233	110	47.2
C×D	56	38	67.9	77	68	88.3	48	28	58.3	48	37	77.1	229	171	74.7
D×E	71	43	60.6	52	32	61.5	53	38	71.7	82	58	70.7	258	171	66.3
E×A	50	34	68.0	44	32	72.7	54	37	68.5	57	41	71.9	205	144	70.2
F×G	92	67	72.8	81	54	66.7	50	40	80.0	86	67	77.9	309	228	73.8
G×F	90	78	86.7	92	55	59.8	72	38	52.8	50	27	54.0	304	198	65.1
Total	475	319	.....	488	320	.....	414	263	.....	450	307	.....	1,827	1,209	66.2

A reduction of the present data in original form by the analysis of variance technique might prove somewhat misleading in view of the fact that percentages have different variances depending on their values and the number of observations on which they are based. A more exact analysis may be made by first transforming the variates to θ values, according to the method proposed by Bartlett (*1*) and Bliss (*2*). This transformation has been made, and the analysis of variance procedure then applied. The analysis of variance based on the weighted θ's corresponding to the observed percentages of fertile ovules is presented in table 3.

TABLE 3.—*Analysis of variance based on weighted θ's for fertility of ovules following self- and cross-pollination*

Type of mating	Source of variance	Degrees of freedom	Sum of squares	Mean square	F	$F_{.05}$	$F_{.01}$
Self-pollination	Between plants	6	14.895378	2.4826	4.365	2.57	3.81
	Between replicates within plants.	21	11.942734	.5687			
	Subtotal	27	26.838112				
Cross-pollination	Between plants	6	13.805651	2.3009	3.245	2.57	3.81
	Between replicates within plants.	21	14.890777	.7091			
	Subtotal	27	28.696428				

The first question of interest to which an answer may be sought is whether the increase in the proportion of fertile ovules following cross-pollination is actually larger than might arise from random sampling alone. Although the number of flowers used and the percentage of fertile ovules observed in the self- and cross-pollinated series vary rather widely, as may be seen in table 2, it is evident from table 3 that the estimated variances for the two groups, i. e., 0.5687 and 0.7091, are similar. The difference, in fact, is nonsignificant, since $F=1.25$, and for significance it would have to exceed 2.08. Consequently, these estimates may be pooled, giving 0.639 as the estimated variance of a single θ value. This corresponds to an estimated standard deviation of 0.709. The weighted mean θ's for the two groups are as follows:

Self-pollination=0.379, based on 2,147 ovules.
Cross-pollination=0.956, based on 1,827 ovules.

The estimated standard deviation between these means, therefore, is 0.025, from which $t=23.1$. As this value of t far exceeds the 1-percent value (2.7) for 42 degrees of freedom, it may be concluded with considerable confidence that the self- and cross-pollinated groups differ significantly in proportion of fertile ovules.

The further question may be asked whether the frequency of fertile ovules is significantly increased by cross-pollination in the case of each plant. Since standard errors for the percentages are not available the significance of the differences must be tested on the basis of the mean θ values. These values for the several plants in the self- and cross-pollinated series are shown in table 4. The largest standard deviation for two mean values for the same plant, but for different series, is clearly less that $0.799\sqrt{\frac{1}{172}+\frac{1}{205}}$, so that an excessive mini-

mal significant difference is found by multiplying this by 2.704 (the 1-percent value for t, with 42 degrees of freedom). The resulting value is 0.223. Referring to table 4, it is seen that the smallest actual difference is 0.278 for plant B. Since this is significant it follows that the increase in percentage of fertile ovules on cross-pollination for each of the other plants is likewise significant.

TABLE 4.—*Mean θ values and percentage of fertile ovules as estimated by inversion from the θ values for the individual plants in the self- and cross-pollinated series*

Plant	Self-pollination			Cross-pollination		
	Total ovules	Weighted mean θ	Estimated fertile ovules	Total ovules	Weighted mean θ	Estimated fertile ovules
	Number		*Percent*	*Number*		*Percent*
A	210	0.423	16.8	289	0.935	64.7
B	172	.479	21.2	233	.757	47.2
C	297	.251	6.2	229	1.054	75.6
D	247	.254	6.3	258	.952	66.4
E	192	.459	19.6	205	.994	70.3
F	586	.445	18.5	309	1.035	73.9
G	443	.354	12.0	304	.950	66.2

A similar procedure may be followed in testing whether the different plants vary significantly in the proportion of ovules becoming fertile under each of the two systems of mating. It is found that the largest minimal 5-percent difference is 0.164 and the smallest minimal 5-percent difference is 0.100. Applying these minimal significant differences, it may be stated that, at the 5-percent level of significance, plants C and D are significantly less fertile than plants A, B, E, and F, and that the latter do not differ significantly from each other. Likewise, D does not differ significantly from C or from G. Furthermore, G is not significantly different from A and F. The exact minimal significant differences between G, on the one hand, and C, E, and B, on the other, are 0.118, 0.135, and 0.141, respectively. Plant G, therefore, does not differ significantly from any of these plants. The comparative fertility relations in the self-pollinated series may be summarized as shown in table 5.

TABLE 5.—*Comparative fertility relations in self-pollinated plants A to G*[1]

Plant	A	B	C	D	E	F	G
A	0	0	+	+	0	0	0
B	0	0	+	+	0	0	0
C	−	−	0	0	−	−	0
D	−	−	0	0	−	−	0
E	−	−	+	+	0	0	0
F	0	0	+	+	0	0	0
G	0	0	0	0	0	0	0

[1] Plant A is significantly more fertile than C, and the latter is significantly less fertile than F. + denotes significantly greater, − significantly less, and 0 nonsignificance.

Proceeding similarly it may be shown that in the cross-pollinated series, plant B is significantly less fertile than the other individuals, and that these latter do not differ significantly from one another.

FERTILITY OF THE OVULES IN RELATION TO POSITION IN THE OVARY

A striking fact which is revealed by histological study of the pistils of alfalfa following self- and cross-pollination is a declining gradient in fertility of the ovules from the apex to the base of the ovary. The observations bearing on this point are summarized in table 6 and figure 2. In the selfed series, about one-third of the ovules in position 1,

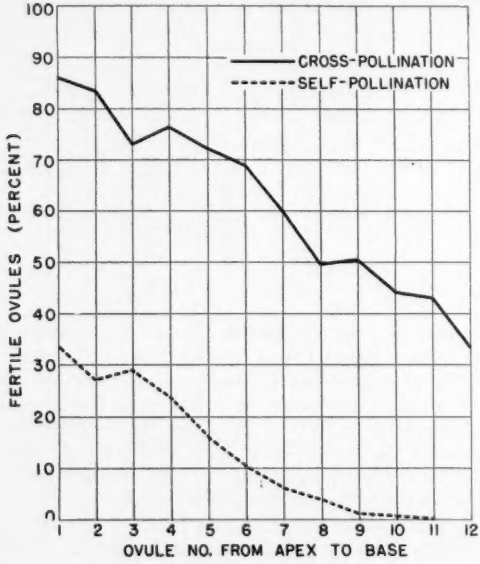


FIGURE 2.—Percentage of fertile ovules according to position in the ovary, following self- and cross-pollination. Averages based on pistils collected at 72, 96, 120, and 144 hours after pollination for seven plants. The apicale ovule is No. 1.

that is, at the apex of the ovary, become fertile. Only about one-half as many in position 5 become fertile, and the frequency declines to zero towards the base.

The fertility gradient follows an almost parallel course in the cross-pollinated series, although the proportion of ovules becoming fertile in each position is much higher than after selfing. In position 1, for example, about 85 percent of the ovules contain fertilized eggs; in position 5 the value has declined to about 72 percent, and at the base of the ovary about one-third of the ovules become fertile.

POLLEN-TUBE GROWTH IN THE OVARIAN CAVITY

The explanation for the difference in the proportion of ovules that become fertile after self- and cross-pollination lies, in part, in the extent of pollen-tube growth following the two types of matings. As mentioned earlier, the position of the ends of only the most advanced pollen tubes within the ovarian cavity can be determined definitely by the technique used. This evidence, nevertheless, is sufficient to

show clearly that restricted pollen-tube growth is a factor affecting fertility after self-pollination.

TABLE 6.—*Fertility of the ovules in relation to position in the ovary following self- and cross-pollination*

[Data based on collections at 72, 96, 120, and 144 hours]

Ovary No.[1]	Self-pollination of ovules			Cross-pollination of ovules		
	Total	Fertile		Total	Fertile	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
1	205	70	34.1	181	156	86.2
2	205	56	27.3	181	152	84.0
3	205	59	28.8	181	133	73.5
4	205	49	23.9	181	139	76.8
5	205	33	16.1	181	131	72.4
6	205	21	10.2	181	125	69.1
7	205	13	6.3	181	109	60.2
8	205	9	4.4	181	90	49.7
9	191	3	1.6	159	80	50.3
10	172	1	.6	131	58	44.3
11	124	0	0	65	28	43.1
12	40	0	0	24	8	33.3
Total	2,167	314	14.5	1,827	1,209	66.2

[1] The apical ovule is No. 1.

The frequency distributions of pistils with reference to the farthest point of pollen-tube advance at 30 hours after pollination in the selfed and crossed series are shown in table 7. Position of the pollen-tube tips is recorded in terms of the particular ovule reached, 1 being the apical ovule. Length is expressed accordingly in "ovule units." The entries in the 0 column relate to pistils in which no pollen tubes were found within the ovary. There are two possible explanations for these cases. Either the pollen tubes were somewhat tardier in development and had not yet emerged from the base of the style, or the pistils had been so injured during castration and pollination that normal growth could not occur. It makes little difference in the conclusions to be drawn later whether these cases are included or omitted. Accordingly, they have been omitted from the summary columns.

It will be observed from table 7 that, in the case of each plant, the pollen tubes are farther advanced after crossing than after selfing. The difference, however, is much larger in some cases than in others. In plant D, selfed, for example, the longest pollen tubes have advanced on an average only slightly beyond the second ovule; whereas, in the cross-pollinated pistils from this individual, the longest tubes have reached the tenth ovule. The longest pollen tubes in plant G, on the other hand, are only slightly longer after hybridization than after selfing. There is little doubt, however, that the difference in length of the longest pollen tubes in the two series is statistically significant, as the comparison in the following paragraph shows.

TABLE 7.—*Frequency distribution of pistils with reference to farthest point of pollen-tube advance at 30 and 48 hours after self- and cross-pollination, respectively*

30 HOURS

Mating	Pollen tubes penetrating to ovule No.—													Total pistils	Mean
	0[1]	1	2	3	4	5	6	7	8	9	10	11	12		
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
A selfed	2		1	2	2	2	1							8	4.0
A × B	1										1	3	1	5	11.0
B selfed		3			1		1	2	2					9	4.8
B × C									3	5				8	8.6
C selfed	1			1	1	3		2						7	5.1
C × D	1			1							1	3	1	6	9.7
D selfed		3	2	2	2									9	2.3
D × E											6			6	10.0
E selfed					1		2	2	1	1				7	6.7
E × A							1		1	2	1			5	8.4
F selfed	2			2	3	3	1							9	4.3
F × G	1				1		1	1	1	1	3			8	8.0
G selfed	5	1	1		3			1	1					7	4.3
G × F	4			2	2	4	4	1						13	5.0

48 HOURS

A selfed					1	2	2	1	1					7	5.9
A × B									1			1	4	6	11.1
B selfed							2			5				7	8.1
B × C									1	4	2			7	9.1
C selfed				1		1	2	2						6	5.7
C × D												4	2	6	11.3
D selfed		1	1	1	2	1								6	3.2
D × E					1						2	4	1	8	10.0
E selfed									3	2	2			7	8.9
E × A									3	3	1			7	8.7
F selfed			2	1	2	5	5	2	3					20	5.4
F × G								1			4	1		6	9.7
G selfed	4			2	2	4	4	1						13	5.0
G × F	1										4	1		5	10.2

[1] Not included in summary columns; relates to pistils in which no pollen tubes were found within the ovary.

The weighted mean length of the longest pollen tubes of the seven plants in the selfed series is 414 ovule units, and the estimated variance is 13.75 ovule units (6 degrees of freedom). In the crossed series the corresponding values are 8.1 and 34.34. Since $F=2.50$, which is less than the 5-percent value, the two variances can be pooled, giving 24.04 as the estimated variance of a single mean. The standard deviation is, therefore, 4.90, and the standard deviation of the difference between the means of the two series is 0.93. It follows that $t=3.978$. Since this value of t exceeds that for the 1-percent point, 3.055, with 12 degrees of freedom, it may be inferred that at 30 hours after pollination the mean length of the longest pollen tubes in the crossed series is significantly greater than that in the selfed group.

An independent series of observations was made on the pollen tubes in the pistils collected at 48 hours after pollination. The data are presented in table 7. The results, in general, point to the same conclusion as was drawn above, namely, that following the application of pollen of an unrelated individual to a given plant the pollen tubes advance farther into the ovary than if the plant in question is self-pollinated. It will be noted, however, that for plant E, the difference, while very small, is in favor of the reverse relation.

With minor exceptions, the average length of the longest pollen tubes is greater at 48 hours than at 30 hours, following both self- and

cross-pollination. The average increase in length in the two series during this interval is 1.5 and 1.3 ovule units, respectively. It is not known whether the tubes continue to elongate beyond this period, for at the time of the next collection, 72 hours after pollination, they have disappeared. At 48 hours in the cross-pollinated series, the average lengths of the longest tubes indicate that the latter are already at o near the base of the ovarian cavity. In the selfed series, on the other hand, they are only slightly beyond the midpoint. Even if it be assumed that the pollen tubes in the selfed pistils continue to grow at the same rate during the 24-hour interval after 48 hours as in the preceding 18-hour interval it is evident that in the case of plants

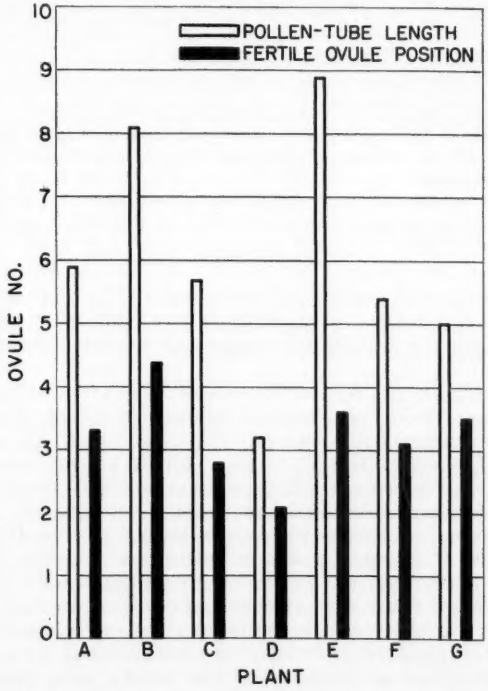


FIGURE 3.—Mean length unit of the longest pollen tubes at 48 hours after pollination in relation to mean position of the fertile ovules following selfing.

A, C, D, F, and G, at least, they would still fall short of reaching the basal ovules.

The mean length of the longest pollen tubes in the selfed series at 48 hours varies from 3.2 ovule units for plant D to 8.9 ovule units for plant E. It is of interest to know whether there is a parallel difference in the distribution of fertile ovules as determined from the collections made at, and following, 72 hours. The mean position of the fertile ovules in each plant has therefore been computed, and the values are presented in table 8 and figure 3 in relation to the corresponding data

for pollen-tube length. If the seven plants are now ranked in ascending order of magnitude for mean length of the longest pollen tubes, and in terms of mean position of the fertile ovules, the arrays shown in table 9 are obtained.

TABLE 8.—*Mean length of longest pollen tubes at 48 hours in relation to mean fertile ovule position at 48, 72, 120, and 144 hours after self-pollination*

Plant selfed	Mean length of longest pollen tubes	Mean position of fertile ovule	Plant selfed	Mean length of longest pollen tubes	Mean position of fertile ovule
A	5.9	3.3	E	8.9	3.6
B	8.1	4.4	F	5.4	3.1
C	5.7	2.8	G	5.0	3.4
D	3.2	2.1			

TABLE 9.—*Array obtained when the seven plants are ranked in ascending order of magnitude for length of pollen tubes and position of fertile ovules*

Character	1	2	3	4	5	6	7
Mean length of longest pollen tube	D	G	F	C	A	B	E
Mean position of fertile ovules	D	C	F	A	G	E	B

Several of the differences upon which the order depends are small and of doubtful statistical significance. Considering all the data, however, a positive relation is suggested between the two sets of observations.

Ovules which are not reached by pollen tubes after selfing obviously cannot become fertile. It does not necessarily follow, however, that the converse relation holds, namely, that ovules to which pollen tubes extend usually become fertile. There is little known concerning this phase of the self-incompatibility problem, so that the present data from alfalfa bearing on the question are of much interest.

It may be computed from the data in table 7 that at 48 hours after self-pollination the longest pollen tubes have reached at least to the apical ovule in 98.5 percent of the 66 pistils examined. The longest pollen tubes likewise extend to the second ovule, at least, in 94 percent of the cases. In other words, 48 hours after self-pollination the two ovules at the apical end of the ovary are reached by at least the longest pollen tubes in nearly all of the pistils. The percentages of fertile ovules in these two positions, on the other hand, are much lower. The value for position 1, as may be seen in table 6, is 34.1 percent, and that for position 2 is 27.3 percent.

In the cross-pollinated series at 48 hours the tips of the longest pollen tubes have passed the two apical ovules in all 45 pistils studied. In marked contrast with the low values after selfing, the percentages of fertile ovules in positions 1 and 2 in this group are 86.2 and 84.0, respectively. If it is shown that the two latter values are significantly higher than the corresponding percentages in the selfed series the operation of a factor affecting fertility in addition to pollen-tube length will have been demonstrated.

TABLE 10.—*Summary of analysis of variance in terms of θ values for fertility of the first two ovules at the apex of the ovary following self- and cross-pollination*

Item	Ovule No. 1		Ovule No. 2	
	Self-pollination	Cross-pollination	Self-pollination	Cross-pollination
Weighted mean of θ's	0.620	1.221	0.536	1.177
Variance (6 degrees of freedom)	.414081	1.026656	.823016	.561734
Ovules, number	205	181	205	181
F ($F_{.05}$=4.28)	2.479	1.465	1.684	2.470
Pooled variance (12 degrees of freedom)	.720368	.692374	.733988	.433804
Standard deviation	.849	.832	.857	.659
Standard deviation of difference of 2 means (12 degrees of freedom)	.087	.085	.087	.067
t ($t_{.01}$=3.055)	6.908	7.541	5.552	8.567

In table 10 are summarized the results of the statistical analysis, the latter again being made on the variates transformed in terms of θ. Since both the t values are much larger than the 1-percent value it is inferred that there is a significantly higher ovule fertility associated with cross-pollination than with self-pollination in each of the first two positions in the ovary. Evidently pollen tubes which reach an ovule after selfing frequently fail to effect fertilization. This conclusion has been confirmed by direct observation of pollen tubes within the ovules after the two types of mating. There is evidence of marked stimulation of the tubes in the vicinity of a micropyle after crossing (pl. 1, *C*). Following self-pollination, on the other hand, pollen tubes frequently pass directly by the micropyle of an infertile ovule without showing any tendency to enter (pl. 1, *B*).

COLLAPSE OF FERTILE OVULES

The development of the fertile ovules arising from the two series of matings was followed up to 144 hours after pollination. The average number of cells in the hybrid embryos (including the suspensor) at this stage is about 18.0 and, in the inbred embryos, about 14.5. Not all the ovules in which development is initiated by fertilization, however, continue growth, even during the comparatively brief period of 6 days after pollination. One of the most significant facts the present investigation has brought to light is that the mortality of the ovules containing inbred embryos and endosperms is much higher than that of the ovules containing hybrid embryos and endosperms. It turns out, therefore, that not only are fewer eggs fertilized following self-pollination but the relative fertility associated with this type of mating is further lowered by the collapse of many of the ovules in which development starts.

The data on frequency of collapsing fertile ovules in the two series as found in the pistils collected at 72, 96, 120, and 144 hours after pollination are summarized in table 11 and figure 4. The means by which this class of ovules may be distinguished have been described earlier by Cooper, Brink, and Albrecht (*6*).

Of the 314 fertile ovules observed in the selfed series, 108, or 34.4 percent, were found to be collapsing within 6 days after pollination. The frequencies for individual plants vary from 20.5 percent for E to 51.4 percent for B. In the cross-pollinated series, on the other hand, only 7.1 percent of the 1,211 fertile ovules recorded were collapsing.

TABLE 11.—*Frequency of fertile ovules collapsing in seven plants after self- and cross-pollination*

[Data based on collections at 72, 96, 120, and 144 hours after pollination]

Plant	Fertile ovules			θ
	Total	Collapsing		
Selfed:	*Number*	*Number*	*Percent*	
A	37	9	24.3	0.515
B	37	19	51.4	.799
C	20	7	35.0	.633
D	17	7	41.2	.697
E	39	8	20.5	.470
F	109	39	35.8	.641
G	55	19	34.5	.628
Total	314	108	34.4	
Crossed:				
A × B	187	13	7.0	.268
B × C	110	5	4.5	.214
C × D	171	13	7.6	.279
D × E	171	16	9.4	.312
E × A	146	9	6.2	.252
F × G	228	14	6.1	.250
G × F	198	16	8.1	.289
Total	1,211	86	7.1	

Furthermore, the percentage of aborting ovules of this class is uniformly low in all the plants. The difference in the mean values is highly significant statistically as the computations in the following paragraph show.

The weighted mean θ for the self-pollinated series is 0.624, based on

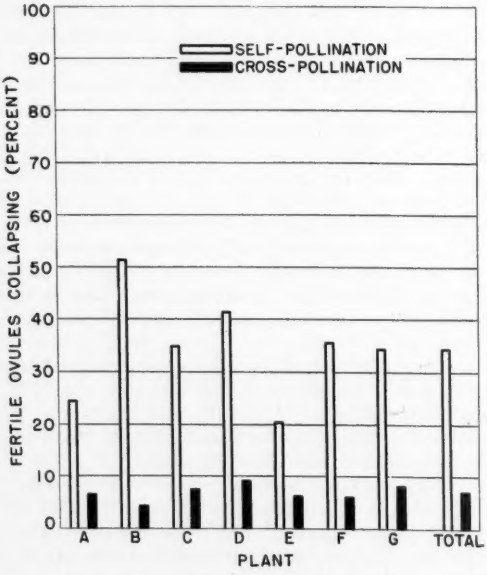


FIGURE 4.—Percentage of fertile ovules collapsing during the first 144 hours after self- and cross-pollination.

314 ovules, and 0.269 for the cross-pollinated group, based on 1,211 ovules. The standard deviation of the difference between the means of the two groups is 0.034. It follows that $t=10.44$, which is much larger than the value of t at the 1-percent point, namely, 3.055.

The relation between position in the ovary and frequency of collapse of fertile ovules is shown in table 12. It will be noted that, after selfing, the proportion of ovules in which development is arrested is comparatively high throughout. There is probably a tendence, however, for the frequency to increase somewhat in the lower positions. In the cross-pollinated series the values are fairly constant down to the ninth ovule, after which a small increase is shown. Uniform gradients similar to those associated with fertilization in the two series are not in evidence here.

TABLE 12.—*Frequency of ovules collapsing in relation to position in the ovary following self- and cross-pollination*

[Data based on collections at 72, 96, 120, and 144 hours after pollination]

Ovule No. [1]	Self-pollination			Cross-pollination		
	Fertile ovules			Fertile ovules		
	Total	Collapsed		Total	Collapsed	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
1	70	18	25.7	156	12	7.7
2	56	16	28.6	152	13	8.5
3	59	17	28.8	133	3	2.2
4	49	20	40.8	139	11	7.9
5	33	12	36.4	131	12	9.2
6	21	10	47.6	125	10	8.0
7	13	10	76.9	111	7	6.3
8	9	4	44.4	90	3	3.3
9	3	1	33.3	80	3	3.7
10	1	0	0.	58	7	12.1
11				28	3	10.7
12				8	2	25.0
Total	314	108	34.4	1,211	86	7.1

[1] The apical ovule is No. 1.

FREQUENCY OF FERTILIZATION AND COLLAPSE OF FERTILE OVULES IN RELATION TO THE PRODUCTION OF MATURE SEEDS

The seven plants used in the experiment formed about 10.5 ovules per pistil, on an average (table 6). It is shown in table 1 that, per flower pollinated, 4.5 mature seeds were formed after crossing and 0.8 seed after selfing. To what extent is the difference between the potential and the actual fertility accounted for by the two factors measured, namely, failure of fertilization and collapse of fertile ovules?

Referring to table 2, it may be seen that 14.6 percent of the ovules became fertile after selfing and 66.2 percent after crossing. Of the ovules which became fertile 34.4 percent containing inbred embryos and endosperms collapsed within 6 days after pollination as compared with 7.1 percent containing hybrid embryos and endosperms (table 11). Considering these two factors alone, the expected average number of seeds per flower is 1.00 and 6.15 for the two respective types of mating (fig. 5). This means that under the conditions of the present

experiment about 98 percent of the difference between the potential and actual fertility following self-pollination and about 67 percent following cross-pollination are accounted for by the lack of fertilization and the collapse of fertile ovules during the first 6 days.

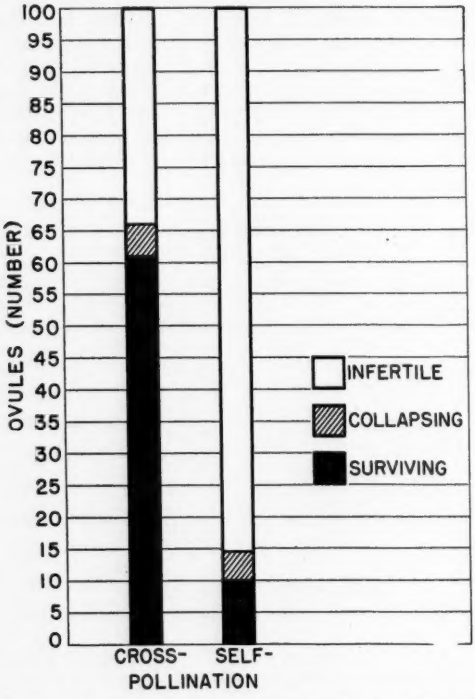


FIGURE 5.—Effect on fertility of failure of fertilization and the collapse of fertile ovules during the first 144 hours after pollination, following selfing and crossing.

DISCUSSION

In an earlier study of fertility in alfalfa in which the effects of self-pollination alone were determined, Cooper, Brink, and Albrecht (*6*) observed that (1) the pollen tubes frequently fail to reach the basal ovules, (2) many ovules remain infertile even though pollen tubes are present, and (3) abortion of fertile ovules is of common occurrence. These findings, which are confirmed in the present investigation, assume added significance in the light of the important additional fact now established that if cross-pollination to an unrelated individual is substituted for selfing the fertility is greatly increased. Crossing was found to raise the proportion of ovules becoming fertile from 15 percent to 66 percent, on an average, and to reduce the abortion of fertile ovules during the first 144 hours after pollination from 34 percent to 7 percent. The higher ovule fertility is found to result from more extensive growth of the pollen tubes within the cavity of the ovary and an increased tendency for the pollen tubes which reach the ovules to

enter the micropyles and accomplish fertilization. These facts make it clear that one of the basic phenomena involved in reproduction in alfalfa is partial self-incompatibility.

About five and one-half times as many mature seeds were formed per flower pollinated after crossing as after selfing in the seven plants used in the present experiment. On the basis of the values given in the preceding paragraph for frequency of fertilization and survival of fertile ovules up to 144 hours after pollination, crossing would be expected to increase the fertility about sixfold. Some additional ovules probably collapse after 144 hours, so that an exact correspondence between the two ratios is not to be anticipated. The fact, however, that the values are of the same order of magnitude lends further weight to the conclusion that the two factors identified are mainly responsible for the increased seed formation on cross-pollination.

Significant differences between individuals were found in the proportion of ovules becoming fertile after self-pollination. The extreme values within the seven plants examined were 6.7 and 21.5 percent. In a previous investigation, Cooper, Brink, and Albrecht (*6*) found one plant in which 43.7 percent of the ovules became fertile after selfing. Doubtless study of larger populations would disclose an even wider spread. The conclusions that self-incompatibility in alfalfa is only partial and varies considerably from plant to plant are in accord with the writers' observations on fertility in larger numbers of individuals being selfed in a breeding experiment by hand-tripping of the flowers. Rarely is a plant found on which no seed is formed after selfing, although the yield is often low. Occasional individuals occur in which the self-fertility is relatively high. It is not known in these latter cases whether it could be increased by applying pollen from unrelated plants. All seven plants in the present experiment, however, showed a statistically significant increase in percentage of fertile ovules on crossing in spite of considerable variation in degree of self-incompatibility.

The abortion of fertile ovules, which occurs so freely following self-pollination, may be a manifestation of self-incompatibility per se, or it may be an inbreeding effect. That is to say, either some of the genes conditioning pollen-tube behavior also may tend to cause ovule collapse, or recessive genes at other loci, adversely affecting early development of the zygote and brought together in homozygous condition by the inbreeding, may be responsible. When the data in table 2 showing the percentage of ovules becoming fertile after self-pollination in the seven plants are compared with those in table 11 showing the frequency of fertile ovules collapsing in the same individuals, no correlation is apparent between the two sets of values. Examination of the data obtained by Cooper, Brink, and Albrecht (*6*) on another series of alfalfa plants leads to the same result. While the evidence is rather limited, it indicates that the collapse of fertile ovules after selfing is more likely an effect of inbreeding than a direct manifestation of self-incompatibility.

The fact is well known to those who have had an opportunity to observe it that alfalfa is highly variable in reproductive behavior. The plant, although blossoming freely, rarely sets seed in many geographical areas in which it is satisfactorily grown as a forage crop. Even in regions where seed is produced commercially, the yield may

fluctuate widely. The reasons for the variability are not yet sufficiently well understood to permit of a general interpretation of this behavior. Meanwhile, however, the implications of partial self-incompatibility may be examined.

The following two deductions of direct significance for an understanding of seed formation in alfalfa may be drawn from the evidence presented above: (1) The potential fertility of a plant will usually, if not always, fall far short of complete realization if self-pollination alone prevails. Under uniform environmental conditions the degree of self-incompatibility varies widely from plant to plant in alfalfa, as it does in many other self-incompatible species (*11*). The fertility of the ovules was markedly higher following cross-pollination, however, in each of the cases in which a direct comparison was made in the present study. The degree of self-incompatibility of a given genotype may also vary according to external conditions judging from the behavior of other self-incompatible species (*11*); but whether the environment can ever shift the reaction of an alfalfa plant to its own pollen so that the usual advantage of crossing disappears remains to be shown.

(2) If, on the stigmas of a given plant, the latter's own pollen is present along with that from an unrelated plant, under conditions favorable for germination, the pollen from the unrelated plant will probably have a large advantage in effecting fertilization. This point is of particular significance for alfalfa since in conformity with the structure of the flower, the stigma usually receives a copious amount of pollen from the anthers encircling it even before the flower opens. The validity of this conclusion rests on (a) the observed fact that, following crossing, the pollen tubes not only grow more rapidly and extend farther into the ovarian cavity than after selfing but they also show a greater tendency to enter the micropyles and effect fertilization in the ovules reached and on (b) the applicability to alfalfa of the rule East and Park (*7*) established for self-incompatible tobaccos that mixed self- and cross-pollination does not increase self-fertilization.

The discovery of partial self-incompatibility brings into sharp relief another phase of the fertility problem in alfalfa, namely, the means by which pollen produced by one plant gains access to the stigma of another. Carlson (*5*) and Hadfield and Calder (*9*) have observed pollen on the standards of untripped flowers in the field. Doubtless much of this is brought from other plants by air currents and insects. When a flower trips, some of the pollen on the standard may be captured by the stigma and cross-pollination thus be effected. The question becomes perplexing, however, when regard is given to the findings of Carlson (*5*) that, under Utah conditions, alfalfa commonly sets seed without tripping of the flowers. Brink and Cooper (*3*) found the same conditions prevailing under unusually hot and dry weather at Madison, Wis., in 1936. Possibily the degree of self-incompatibility is lowered by the conditions of growth in these cases and much self-fertilization occurs. It seems more probable, however, that pollen from other plants gains access to the stigmas of untripped flowers by some means not yet described. Brink and Cooper (*3*) observed pollen germinating within untripped flowers and pollen tubes entering the styles, even in the late bud stage. Almost certainly the pollen functioning in the latter cases was from the same respective flowers. Conceivably, this

early self-pollination initiates wilting of the corolla and stimulates development of the pistil so that the stigma becomes exposed to pollen from other flowers. Since after selfing, the pollen tubes grow more slowly and fail to fertilize the eggs in many of the ovules, the pollen from other individuals might largely prevail in fertilization in spite of its later start. Obviously, this question requires further investigation.

SUMMARY

Following self-pollination under greenhouse conditions only 14.6 percent of the ovules of alfalfa become fertile, on an average, as compared with 66.2 percent after cross-pollination on the same plants.

Individuals vary significantly in the proportion of ovules that become fertile after selfing. The variability is less after cross-pollination.

Most of the fertile ovules resulting from self-pollination are in the apical half of the ovary. A rather uniform gradient in fertility occurs from the apex downwards, the ovules in the lowermost positions rarely containing fertilized eggs.

A parallel gradient in fertility is found after cross-pollination, although the proportion of ovules becoming fertile in each position is much higher than after selfing.

Pollen-tube growth, as determined by the position of the tips of the longest pollen tubes at 30 hours and at 48 hours after pollination, is more rapid in cross matings than after selfing.

It is probable that the basal ovules in the ovary are seldom reached by pollen tubes after selfing. Following cross-pollination the tubes usually grow to the base of the ovary.

In the self-pollinated series there appears to be a positive, although not high, correlation between mean length of the longest pollen tubes and mean position of the fertile ovules from plant to plant.

The low fertility of the ovules following self-pollination is due only in part to restricted pollen-tube growth. Direct observation confirms other, more general, evidence adduced that frequently after selfing the pollen tubes pass directly by the micropyles of ovules containing unfertilized eggs.

About one-third of the fertile ovules in the selfed series were found to be collapsing in the pistils collected at 72 to 144 hours after pollination as against only 7.1 percent in the crossed series.

Taking into consideration the differentials both in proportion of ovules becoming fertile and proportion of fertile ovules collapsing, the net fertility at 144 hours after pollination is about six times as high in the crossed series as in the selfed series.

The partial incompatibility associated with self-pollination and the high incidence of abortion of ovules containing inbred embryos during the first 6 days after pollination appears sufficient to account for most of the difference in seed production following selfing and crossing.

Under the conditions of the present experiment about 98 percent of the difference between the potential and actual fertility following self-pollination and about 67 percent following cross-pollination are accounted for by the frequency of fertilization and the collapse of fertile ovules during the first 6 days.

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SOME FACTORS IN LAMB PRODUCTION ASSOCIATED WITH SIZE AND TYPE IN MUTTON SHEEP[1]

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INTRODUCTION

Among the different breeds of sheep there are marked differences in scale, weight, conformation, and fleshing qualities as well as in wool production. The factors of rapid growth and desirable mutton type receive primary consideration from most growers of the mutton breeds, whereas breeders of fine-wool sheep place great emphasis on the fleece. Western range men endeavor to maintain heavy wool-producing qualities in the ewe flock with a predominance of fine-wool breeding while raising an acceptable lamb for slaughter or feeding purposes by the use of various breeds of mutton-type rams. Farmers in the Central West desiring to purchase breeding ewes usually find greater numbers and more uniform selection among western ewes than among native ewes, and consequently they are confronted with the problem of the type and breed of ram to use.

Considerable data are available on the various methods of breeding for early spring lambs in the East (*7*, *9*, *16*),[3] for early California lambs to be shipped to New York and other eastern markets (*13*), and for the production of the large western lamb crop (*1*, *8*, *10*, *11*); yet there is little information on certain phases of lamb production in the Central West[4] (*2*, *3*). Few records have been made of comparative feed consumption of large and small ewes and their lambs in the course of the year[5] (*9*, *10*, *15*), as practically all data are computed on a per lamb or per ewe basis with little or no regard to returns for feed consumed. In fact, as Hunt (*9*) points out in some of the tests referred to, if feed consumption were on the approximate basis of weights of the ewes, the interpretation of results quoted by the authors might be doubtful, or in many cases reversed. Records of carcass yields, including boned edible meat, for various types and sizes of lambs, are also lacking. Because of this scarcity of data a study was made at the Illinois Agricultural Experiment Station in which various factors in lamb production associated with size and type of sheep were considered.

[1] Received for publication October 21, 1939. This paper is part of a thesis presented to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of doctor of philosophy. The work herein described was done at Illinois while the author was on leave of absence from the Michigan station. The paper is presented as a contribution from the Illinois Agricultural Experiment Station.

[2] The author is especially indebted to Prof. H. P. Rusk of the University of Illinois for suggestions and guidance in the conduct of this study. Grateful acknowledgment is also made for the generous cooperation and assistance in the experimental procedure by members of the following divisions in the Department of Animal Husbandry, Animal Nutrition, Meats, and Sheep Husbandry. Additional assistance in certain phases through the Work Projects Administration Project No. 80057 is likewise appreciated.

[3] Italic numbers in parentheses refer to Literature Cited, p. 486.

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[5] See footnote 4.

OBJECTS OF INVESTIGATION

In these tests one of the largest mutton breeds, the Hampshire, was compared with one of the smallest mutton breeds, the Southdown. Other tests were made in which rams of the two breeds were mated with similar groups of western ewes. In these cases, rams of a breed intermediate in size, the Shropshire, were also used.

The objects of the investigation may be summarized as follows: (1) To determine the comparative total feed requirements for the production of market lambs from sheep of large and small mutton breeds; (2) to compare the digestive efficiency of sheep of the different groups; (3) to study methods of measuring meat values in lambs; (4) to compare yield and quality of meat from lambs of the two groups; (5) to compare the efficiency of rams of different size and breed for siring market lambs from grade western ewes under Central States conditions; and (6) to compare fine-wool type and crossbred-type western ewes under such conditions.

TESTS WITH PUREBRED LAMBS OF LARGE AND SMALL MUTTON BREEDS

Twenty Southdown ewes were purchased in Kentucky, 10 from each of two breeders. Five ewes were 2-year-olds and the others were yearlings. Twenty Hampshire ewes were purchased at the stockyards in Evansville, Ind., where they had been sent for sale from a ranch in Utah. They were 3 years old. The ewes in each group were purebred and were considered by members of the Animal Husbandry Department to be typical of the two breeds. The average weight of the Southdown ewes was 109 pounds and that of the Hampshire ewes 153 pounds. The lightest Southdown ewe weighed 94 pounds and the heaviest 123, while the lightest Hampshire weighed 140 pounds and the heaviest 175.

The ewes were mated with purebred rams of the respective breeds, each of which had proved to be a good sire in the university flock. The Southdown ram was 6 years old, weighed 149 pounds, and was considered by sheepmen to be typical of the Southdown breed. The Hampshire ram was similar in age, of good type, and weighed 230 pounds.

METHOD OF PROCEDURE

Feed records were kept on each group of ewes from September 7, when breeding started, until the lambs were slaughtered. Feed for the dry period, to complete the yearly record after the lambs were slaughtered, was estimated on the basis of that required to maintain body weight during the first 8 weeks of pregnancy. Corn silage and alfalfa hay constituted the ewe ration, except during the latter part of pregnancy and during the nursing period, when grain was added. A mixture of bonemeal, limestone, and salt was available.

All lambs were born between January 30 and March 7, 1938, the average birth date of the Southdown lambs and of Hampshire single lambs being February 17, and that of Hampshire twin lambs February 14. For various reasons, such as death of ewes or of lambs, injury, failure of milk supply, or slaughter of finished lambs, it was necessary to remove individuals from time to time; and the feed was calculated

on the per head basis for each 14-day period. There were 14 Hampshire twins, 7 single lambs, and 14 Southdown single lambs on which complete feed data were obtained by lots.

A creep was arranged in each pen so that the lambs could go to the feed as they desired, although it was not allowed to accumulate before them. The same mixture, composed of corn, oats, and linseed meal, was fed each lot. The feed mixture was changed from time to time by increasing the corn content and later adding some cut alfalfa hay. The lambs ate some feed with the ewes in addition to that offered in the creep. Since all rations were from the same feeds in similar proportions, repeated chemical analyses were not made; however, one analysis was made of representative feeds used. The total digestible nutrients were estimated from Morrison's tables (*14*) for the kinds of feed used. This was done to convert all feeds to one unit of measure for approximate comparisons.

Time of reaching market finish, and average monthly changes in lamb prices by grades for 3 years, based on reports of the Bureau of Agricultural Economics of the United States Department of Agriculture, were considered in connection with the other factors. Previous shearing dates for the ewes were unknown, making study of wool production impossible.

The lambs were slaughtered when they reached a choice market finish, as judged by appearance and handling qualities. Physical and chemical analyses of the carcasses were made for two reasons, namely, to determine whether slaughter had been done when the two groups of lambs were similar in finish and to measure differences in the yield and quality of meat. Content of the digestive tract and weights of body parts were obtained at slaughter. The various carcass cuts were made and the right half of each carcass was separated with the knife into bone, lean, and fat. Some measurements were made to determine the relation of size of eye muscle in the loin and rib cuts to quantity of lean in the carcass. Measurements of color of lean in the rib eye were made with a spectrophotometer, showing the dominant hue or primary color, with its degree of purity and brightness. Chemical analyses were made of dry matter, ether extract, and total nitrogen in the total lean and total fat of the half carcass. Measurements of color were made by the Division of Meats and chemical analyses by the Division of Animal Nutrition, of the Univeristy of Illinois.

No data could be found to prove or disprove, among other things, the frequently expressed opinion that larger sheep handle roughages more efficiently than smaller sheep. Consequently, trials were conducted with six sheep of each breed 12 months old to compare efficiency in digestion of a full-fed ration of equal parts of grain and hay. The average weight of the Southdown sheep was 82.7 pounds, and that of the Hampshires 135.3 pounds.

Digestion data were also obtained with 20 pairs of Hampshire-sired and Southdown-sired grade lambs from this experiment. These lambs were fed at or slightly above maintenance level on approximately equal parts of grain and hay. The weights of the pairs were more nearly alike than in the test with purebred sheep. These tests were conducted in the animal nutrition laboratory in digestion crates similar to those described by Forbes (*6*). Collections were made over 10-day periods after preliminary periods of 10 days.

RESULTS

Statistical analyses of data were made according to Fisher's (*5*) method for small numbers, in which values for P of 0.01 or less are considered highly significant and values between 0.01 and 0.05 are considered significant.

Purebred Hampshire ewes, averaging 153 pounds in weight when bred, maintained their weights in a similar manner to purebred Southdown ewes averaging 109 pounds, when fed 50 percent more feed than was allowed the Southdown ewes during the first 8 weeks of pregnancy. Hampshires showed a stronger tendency to increase feed consumption and to gain in body weight when full-fed similar rations during the remainder of the pregnancy period, although fatness was similar in the two groups. Average weights before lambing were 170 and 112 pounds respectively, or approximately 50-percent greater weight for the Hampshire ewes than for the Southdown ewes. Among the 20 Hampshire ewes, there were born later 9 sets of twin lambs and 1 set of triplets, whereas there were only 2 sets of Southdown twins from the 20 Southdown ewes.

For maintenance during the year and for developing a desirable market lamb weighing 86 pounds, a Hampshire ewe and single lamb ate about 50 percent more alfalfa hay and corn silage and practically the same quantity of concentrates as did a Southdown ewe and lamb in developing a lamb weighing 66 pounds (table 1).

TABLE 1.—*Feed consumed by purebred ewes and lambs during various periods and for the year*

Lambs and period	Alfalfa hay	Corn silage	Shelled corn	Oats	Wheat bran	Linseed meal
Southdown ewe (average of 14; single lamb):	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Breeding and pregnant, 168 days	241	304	.....	21	.....	.....
Nursing, 156 days	214	329	137	.....	.....	22
Dry, 41 days	62	62	.....	.....	.....	.....
Total for the year (ewe)	517	694	137	21	.....	22
Lamb	27	.....	70	37	4	14
Total for ewe and lamb	544	694	207	58	4	36
Hampshire ewe (average of 7; single lamb):						
Breeding and pregnant, 168 days	364	471	.....	35	.....	.....
Nursing, 110 days	220	432	160	.....	.....	25
Dry, 87 days	196	196	.....	.....	.....	.....
Total for the year (ewe)	780	1,099	160	35	.....	25
Lamb	14	.....	43	34	5	11
Total for ewe and lamb	794	1,099	203	69	5	36
Hampshire ewe (average of 7; twin lambs):						
Breeding and pregnant, 168 days	364	471	.....	35	.....	.....
Nursing, 152 days	330	523	203	.....	.....	33
Dry, 45 days	101	101	.....	.....	.....	.....
Total for the year (ewe)	795	1,095	203	35	.....	33
Lamb (1)	32	.....	88	51	6	19
Total for lamb and one-half ewe total for the year	429	547	189	68	6	36

The calculated total digestible nutrient content of the total feeds eaten per ewe and lamb was approximately 30 percent higher for the Hampshire ewe and single lamb than for the Southdown ewe and lamb, as indicated in table 2.

TABLE 2.—*Total digestible nutrient requirement for purebred ewes and lambs, per lamb and per unit of body weight, including the ewe's requirement for the year*

Item	Average feet-lot final weight [1]	Total digestible nutrients per—			
		Lamb	Hundredweight of final weight	Hundredweight of chilled carcass	Hundredweight of carcass lean
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*
Southdown single	65.57	643	980	1,960	3,608
Hampshire single	85.93	848	987	2,014	4,143
Hampshire twin	84.43	551	653	1,396	2,556
Average Hampshire lamb from—					
1 single, 2 twins, or 150 percent crop	84.93	650	764	1,602	3,085
2 singles, 1 twin, or 120 percent crop	85.43	749	876	1,808	3,614

[1] Averages of 14 Southdowns, 7 single, and 14 twin Hampshires.

The average slaughter weight of Southdown lambs at an average age of 164 days was approximately 20 pounds lighter than that of Hampshire lambs when 117 days of age, although the finish was fully as good if not slightly better in the Southdowns.

More hay and silage were required per hundred pounds of single Hampshire lamb produced, on account of the much larger amounts eaten by the ewes, especially during the dry period; while more concentrates were required per hundred pounds of Southdown single lamb, on account of the longer nursing period of the ewes and growth period of the lambs.

The estimated total digestible nutrients required for each hundred pounds of final feed-lot lamb weight were practically the same for Southdown and Hampshire single lambs (table 2).

Slightly higher yield of dressed carcass for Southdown lambs gave them about a 3-percent advantage in total digestible nutrient requirement for each hundred pounds of dressed carcass as compared with the Hampshire single lambs.

A still higher yield of lean meat in the carcass gave the Southdown lambs a 13-percent advantage over the Hampshire singles in digestible nutrient requirement per hundred pounds of lean meat.

A Hampshire twin lamb's feed and its half of the mother's yearly feed was only slightly lower in concentrates but only half as high in alfalfa hay and silage as that of the Hampshire single lamb and its mother. Slightly less concentrates and about 20 percent less of the roughages were required than in the case of the Southdown single lamb and its mother.

The estimated total digestible nutrient content of the total feeds eaten per Hampshire ewe and single lamb was approximately 55 percent greater than that of the twin lamb and its half of the mother's feed. Nutrient requirement per hundred pounds of finished lamb weight was 50 percent greater for either the Hampshire or Southdown singles than for the Hampshire twins.

On the basis of the dressed carcass yield, Hampshire singles required 45 percent more total digestible nutrients than twins. On the basis of lean-meat yield, Hampshire singles required 62 percent more nutrients than twins, while Southdown singles required 41 percent more than the Hampshire twins.

On the basis of total digestible nutrient requirement per hundred pounds of lean meat, a Hampshire lamb crop of one pair of twins to each four single lambs, or a 120-percent lamb crop, would be necessary to equal a Southdown lamb crop of all single lambs. A summary of unpublished data from the University of Illinois sheep flock over an 18-year period shows that for each 100 Hampshire ewes, 148 lambs were born; while for each 100 Southdown ewes, 122 lambs were born; or for each 100 Southdown lambs, 122 Hampshire lambs were born.

Hampshire single lambs born in February were ready for market in June at an average age of 117 days, while Hampshire twin lambs and Southdown single lambs born at the same time were not ready until middle or late July, at ages of 155 and 164 days, respectively. Average feed-lot weights were as follows: Southdown singles, 65.57 pounds; Hampshire singles, 85.93; and Hampshire twins, 84.43.

The average Southdown lamb was graded Top Choice both on foot and in carcass, while the average Hampshire was Low Choice in grade in both cases.

According to Chicago lamb quotations by grades for the years 1936, 1937, and 1938, as shown in table 3, the average price advantage of Low Choice Hampshire single lambs in June was about $1 per hundred pounds as compared with Top Choice Southdown single lambs in late July or early August. Low Choice Hampshire twin lambs ready for market about the same time as Southdown single lambs were worth slightly less than the Southdown lambs because of lower grade; and they were worth fully $1.08 per hundred pounds less than Hampshire single lambs because of lower prices in July than in June.

TABLE 3.—*Chicago prices per hundred pounds of live lambs by grades, expressed as monthly averages for 1936–38*

Month	Choice	Good	Medium	Plain
May	$11.37	$10.86	$10.13	
June	11.26	10.74	9.89	$8.44
July	10.18	9.63	8.68	7.50
August	9.95	9.48	8.52	7.27
September	9.64	9.16	8.13	6.86
October	9.23	8.82	7.85	6.60
November	9.33	8.91	7.96	6.68

The greater the proportion of twins among the Hampshires, the greater the saving in feed per hundred pounds of lamb, although a lower average selling price would be expected since fewer single lambs were ready for the early market.

Lambs born in March instead of February and growing at a similar rate would be ready for market a month later than those in this experiment. In such cases the price of Hampshire singles in July, on the basis of prices during the last 3 years, would be about 20 cents higher than that of Southdown singles in August, as compared with $1 difference in price a month earlier.

Higher yield of boneless meat from each hundred pounds of live lamb made Southdown lambs worth $11.13 when Hampshire lambs were worth $10 per hundred pounds. This would be fully as great an advantage for Southdown lambs in a discriminating market as the price advantage for Hampshire lambs if sold on the June market.

The content of the digestive tract in the feed-lot was 2.73 percent more for Hampshire lambs than for Southdowns on the basis of empty body weight. Other offal and byproducts at slaughter were also higher for Hampshire lambs, resulting in 2 percent more warm carcass yield for Southdowns on the basis of empty weights, and 3.04 percent more chilled carcass on the basis of slaughter weight. Both of these differences were highly significant according to Fisher's method for small numbers.

Numerical differences in average percentages of retail cuts as proportions of the carcass were small, ranging from 0.03 to 0.61 percent, and were not statistically significant. Average total percentages of the four major cuts, leg, loin, rib, and shoulder, were 73.40 and 73.26 for Southdowns and Hampshires, respectively.

Average content of bone in the right half-carcass was 3.28 percent higher numerically in the Hampshire carcasses than in the Southdowns, a highly significant difference. Bone content of each retail cut and bone trim in the carcass were significantly higher for Hampshire lambs. (Table 4).

TABLE 4.—*Statistically significant differences in composition of carcasses and cuts from purebred lambs*

Portion of carcass	Average composition		Difference in favor of Southdown	Standard error of difference	t value [1]	Significance
	Southdown	Hampshire				
	Percent	*Percent*	*Percent*			
Bone in right half of carcass	13.05	16.33	−3.28	0.5638	5.818	High.
Bone in trimmed right-side cuts:						
Leg	12.96	16.53	−3.57	.4807	7.425	Do.
Loin	10.33	12.51	−2.18	.7405	2.95	Do.
Rib	16.94	20.13	−3.19	1.074	2.97	Do.
Shoulder	13.61	16.94	−3.33	.751	4.44	Do.
Breast	12.58	16.73	−4.15	.709	5.85	Do.
Shank	31.44	40.48	−9.04	1.333	6.78	Do.
Bone trim as proportion of carcass	1.51	1.89	−.38	.0697	5.452	Do.
Lean meat as proportion of trimmed right-side cuts:						
Leg	66.55	64.46	2.09	1.017	2.055	Significant.
Rib	52.67	49.15	3.52	1.619	2.17	Do.
Shank	49.99	46.44	3.55	1.131	3.14	High.
Selected lambs: [2]						
Lean meat as proportion of:						
Right-half carcass	55.89	53.63	2.26	.936	2.41	Significant.
Trimmed right leg	67.20	65.33	1.87	.780	2.40	Do.
Fat as proportion of right shank	16.70	11.80	4.90	1.009	4.856	High.
Lean and fat as proportion of right half of carcass	80.50	76.79	3.71	.5386	6.888	Do.

[1] FISHER (*5, table IV*).
[2] 10 Southdown and 15 Hampshire lambs intermediate in fatness.

Yield of boneless meat was, conversely, higher for Southdown lambs, averaging 3.71 percent above that in Hampshire lambs—a highly significant difference.

Average content of mechanically separable lean was 1.9 percent higher in the half carcass of Southdown lambs, but the difference was

was not significant when all lambs were included. When extremely thin and fat carcasses were excluded, averages of 10 Southdowns and 15 Hampshires gave a difference of 2.26 percent, which is significant. Average content of lean as percent of the trimmed cut was significantly higher for Southdowns in the leg, rib, and shank only when all lambs are considered.

Average fat content separated with the knife was not significantly different in the half carcass or in any cut except the foreshank. Southdown half carcasses averaged 1.81 percent higher in fat, and they were higher in all cuts except carcass trim, loin, and rib. If fat trim were added to the other carcass fat, Southdowns would be only 1.2 percent higher in fat content than Hampshires.

Average area of eye muscle on the anterior surface of the loin cut as measured with a planimeter was not significantly different for 11 Southdown lambs and 12 Hampshire twin lambs measured.

Correlations were high between area of loin eye muscle and weights of each of the following parts among Hampshire lambs, or Hampshire and Southdown lambs together: Right half carcass, lean in the half carcass, loin eye muscle, loin lean, and rib eye muscle. Correlation of area of loin eye with weight of Southdown loin eye, and lean in the half carcass, and with Hampshire rib lean was significant in each case, but not highly so.

Color readings on the rib eye muscle by means of a spectrophotometer, giving wave lengths for hue in millimicrons, and percentages of brightness and purity, showed no differences between the two groups of lambs, but indicated a relatively bright pinkish red color as seen by the eye.

Dry substance and ether extract each averaged slightly higher in the boneless meat of Southdown carcasses than in that of Hampshire carcasses, but the differences were not statistically significant. When divided into content of lean and of fat separately, there were significant but small differences in total nitrogen of the lean, and in dry substance and ether extract of the fat, the difference favoring Southdowns in each case. Both physical and chemical analyses of the carcasses indicated a similar degree of finish in the Southdown and Hampshire lambs.

Apparent digestibility of a full ration of equal parts of corn and hay by six pairs of purebred Southdown and Hampshire yearling sheep was similar except for the total nitrogen, as shown in table 5. An average of 60.2 percent of the total nitrogen was digested by the Hampshires as compared with 55 percent by the Southdowns, a highly significant difference. Each Hampshire excelled its pair mate in the use of nitrogen and also in most cases in the use of other nutrients. Average coefficients of digestibility for dry matter, crude fiber, and gross energy were higher for the Hampshires, but differences were not significant. Feed consumption by three pairs of lambs when full-fed during a 3-week preliminary period was found to be on the basis of the three-fourths power function of the body weight. These results formed the basis of feed allotment and the other six lambs were paired satisfactorily on a similar basis.

TABLE 5.—*Summary of digestion coefficients for purebred lambs 12 months old, full-fed*

Breed, number, and sex of animals	Weight of sheep	Average daily feed	Digestion coefficient of—			
			Dry matter	Gross energy	Crude fiber	Total nitrogen
Southdowns:	*Pounds*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
A–1, ram	71	840	69.8	68.6	42.8	54.7
B–3, ewe	80	920	71.0	70.2	39.7	56.6
C–5, ewe	88	988	69.6	68.4	36.0	55.2
D–7, ewe	71	780	70.3	69.2	38.3	57.2
E–9, ewe	91	944	69.3	68.0	39.1	54.4
F–11, ewe	95	852	67.0	65.6	33.1	52.0
Average	82.7	887	69.5	68.3	38.2	55.0
Hampshires:						
A–2, ram	155	1,512	73.2	71.8	43.2	64.1
B–4, ewe	132	1,340	72.1	70.7	43.9	58.4
C–6, ewe	143	1,420	70.8	70.0	40.0	58.9
D–8, ewe	118	1,144	69.9	68.2	38.2	61.9
E–12, ewe	139	1,296	70.4	68.9	40.5	59.9
F–10, ewe	125	1,048	68.9	67.8	37.6	58.3
Average	135.3	1,293	70.9	69.6	40.6	60.2
Mean difference	52.6	406	1.38	1.23	2.4	5.23
One-half *P* by Fisher's method for small numbers.			.07	.125	.097	.005
Significance			None	None	None	High

There was no significant difference between Southdown-sired grade lambs and Hampshire-sired lambs from similar ewes in ability to digest a maintenance or slightly above maintenance ration of equal parts of grain and hay. These 20 pairs of lambs were fed on the basis of the 0.74 power of body weight, which is in line with suggestions by Kleiber (*12*) and by Brody, Proctor, and Ashworth (*4*). The Southdown-sired lambs averaged 79.5 and the Hampshire-sired lambs 69.5 pounds in weight, which is contrary to expectation in the case of purebreds of the two breeds. While the heavier lambs tended to be more efficient, the differences were not statistically significant.

TESTS WITH LAMBS FROM GRADE WESTERN EWES

In the Dixon Springs project, located in Pope and Johnson Counties in southeastern Illinois, a program supervised by the University of Illinois in cooperation with the United States Government is being developed in an effort to establish pasture grasses on the worn-out land typical of some of that in several States. A flock of 360 western ewes was available in this area for the production of lambs to be raised under southern Illinois conditions.

One group of 112 2-year-old ewes was apparently of predominant Merino or Rambouillet breeding and originated in Montana. Another group of 248 white-faced ewes originating in the State of Washington had the appearance of sheep sired by long-wooled rams and out of fine-wooled ewes. The crossbred ewes averaged 104 pounds in weight in September as yearlings, as compared with 92 pounds for the 2-year-old fine-wooled ewes.

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METHOD OF PROCEDURE

The fine-wooled and crossbred ewes were in each case divided into three lots as nearly uniform as possible according to weight, conformation, fleshing, fleece, and other characteristics commonly considered in breeding ewes. Throughout the experiment all ewes were either fed and pastured together as one flock or else the rations and management were as nearly identical as possible. Rams of the three breeds—Hampshire, Shropshire, and Southdown—were placed with the respective lots of ewes on September 10, 1937. The three Hampshire rams were yearlings, weighing 158, 160, and 162 pounds; the Shropshires were also yearlings and weighed 135, 144, and 159 pounds; one of the three Southdowns was an aged ram weighing 160 pounds and the other two were yearlings weighing 118 and 132 pounds, respectively.

On July 25, 1938, 72 percent of all wether lambs were sold on the Chicago market. The remaining wethers were weaned shortly afterward and fed grain on pasture until marketed on October 17. The ewe lambs were weaned August 8, and were weighed and graded at the farm by a lamb salesman from a Chicago livestock commission firm. They were not marketed, but market weight was estimated from the shrink on the wether lambs.

Complete data concerning weights and live and carcass grades were obtained on the wether lambs on the market and in Armour's and Swift's packing houses. Each lamb carcass was graded by the supervisor of Federal meat grading for the Chicago district. Since all wether lamb carcasses were graded more carefully by the Federal supervisor than it was possible to do with the live lambs, carcass grades for the wethers were used in computing values. Only single lambs were used in the data analyzed.

Monthly market prices of live lambs by grades at Chicago, as reported by the Bureau of Agricultural Economics of the United States Department of Agriculture for the years 1936, 1937, and 1938, (shown in table 3) were used in assigning values. There was a sharp decline in price from June to July and a gradual fall until October, with a slight rise in November. There was a similar trend for each grade of lambs, resulting in a similar spread in prices between grades from month to month. Average value per lamb in each group was obtained from the sum of all lambs by grades. Those of each grade were valued at the price for the month when graded or marketed.

RESULTS

Time of marketing, weight, and grade were factors determining the value per lamb. Average birth dates of all groups of lambs, according to breeding or according to time of marketing, were similar. More Hampshire- and Southdown-sired wether lambs were ready for the higher market in July than of Shropshire-sired wethers, the percentages being 76, 79, and 61, respectively.

Distribution of lambs by farm weights is indicated in table 6 and by grades in table 7. The Hampshire-sired lambs were characterized by slightly higher weights, while Southdown lambs were outstandingly highest in grade. The distribution of lambs by weights and by grades was similar for those of each sex. The weights of wether lambs were slightly higher than those of ewe lambs on account of the marketing of

some wether lambs later after further feeding, but the results among lots were not materially changed thereby.

TABLE 6.—*Distribution of lambs from grade western ewes by farm-weight classes*

Description of lambs	Lambs in weight class—							Average weight of lambs in group
	90 or more pounds	85–89 pounds	80–84 pounds	75–79 pounds	70–74 pounds	65–69 pounds	64 pounds or less	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Pounds*
Wether lambs from—								
Crossbred ewes:								
Hampshire sire	3	5	7	4	8	4	----	78.74
Shropshire sire	----	2	9	5	5	2	3	75.15
Southdown sire	2	4	10	2	10	1	3	77.19
Fine-wooled ewes:								
Hampshire sire	1	3	3	3	----	----	----	83.0
Shropshire sire	4	----	3	7	4	2	3	76.78
Southdown sire	----	3	5	1	3	3	1	76.3
All ewes:								
Hampshire sire	4	8	10	7	8	4	----	79.8
Shropshire sire	4	2	12	12	9	4	6	75.9
Southdown sire	2	7	15	3	13	4	4	76.9
All rams:								
Crossbred ewes	5	11	26	11	23	7	6	77.18
Fine-wooled ewes	5	6	11	11	7	5	4	77.9
Ewe lambs from—								
Crossbred ewes:								
Hampshire sire	----	3	3	2	5	4	1	75.1
Shropshire sire	----	2	5	6	7	2	3	74.3
Southdown sire	----	1	4	4	6	1	4	73.5
Fine-wooled ewes:								
Hampshire sire	1	5	----	4	2	3	1	76.4
Shropshire sire	----	----	----	2	6	5	1	70.4
Southdown sire	----	----	----	4	5	5	----	71.1
All ewes:								
Hampshire sire	1	8	3	6	7	7	2	75.7
Shropshire sire	----	2	5	8	13	7	4	72.9
Southdown sire	----	1	4	8	11	6	4	72.5
All rams:								
Crossbred ewes	----	6	12	12	18	7	8	74.3
Fine-wooled ewes	1	5	----	10	13	13	2	72.8

TABLE 7.—*Distribution of lambs from western ewes by market grades*

Market grades	Choice	Good	Medium	Plain	Cull	Total
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Carcass grades of wether lambs from—						
Crossbred ewes:						
Hampshire sire	1	18	7	5	----	31
Shropshire sire	1	17	5	3	----	26
Southdown sire	22	9	1	----	----	32
Fine-wooled ewes:						
Hampshire sire	1	7	1	1	----	10
Shropshire sire	----	11	11	----	1	23
Southdown sire	6	10	----	----	----	16
All ewes:						
Hampshire sire	2	25	8	6	----	41
Shropshire sire	1	28	16	3	1	49
Southdown sire	28	19	1	----	----	48
All rams:						
Crossbred ewes	24	44	13	8	----	89
Fine-wooled ewes	7	28	12	1	1	49
Live grades of ewe lambs from—						
Crossbred ewes:						
Hampshire sire	----	5	9	2	2	18
Shropshire sire	5	14	5	----	1	25
Southdown sire	6	10	3	1	----	20
Fine-wooled ewes:						
Hampshire sire	2	8	3	2	1	16
Shropshire sire	2	8	4	----	----	14
Southdown sire	3	9	1	1	----	14
All ewes:						
Hampshire sire	2	13	12	4	3	34
Shropshire sire	7	22	9	----	1	39
Southdown sire	9	19	4	2	----	34
All rams:						
Crossbred ewes	11	29	17	3	3	63
Fine-wooled ewes	7	25	8	3	1	44

TABLE 8.—*Market weights and value per animal of lambs from grade western ewes*

Wether and ewe lambs	Lambs	Average weight per lamb	Average value per lamb	Wether and ewe lambs	Lambs	Average weight per lamb	Average value per lamb
From crossbred ewes:	*Number*	*Pounds*	*Dollars*	From all ewes:	*Number*	*Pounds*	*Dollars*
Hampshire sire	49	73.06	6.37	Hampshire sire	75	73.56	6.52
Shropshire sire	51	70.53	6.46	Shropshire sire	88	70.39	6.38
Southdown sire	52	71.62	6.88	Southdown sire	82	70.93	6.80
From fine-wooled ewes:				By all rams:			
Hampshire sire	26	74.50	6.79	Crossbred ewes	152	71.72	6.58
Shropshire sire	37	70.10	6.27	Fine-wooled ewes	93	71.25	6.54
Southdown sire	30	69.73	6.66				

The records for all lambs are combined in table 8 to show average market weight and value per lamb by lots. Less spread in live grades and values among ewe lambs than among wether lambs, which were graded in the carcass, tended to narrow the spread in value among all lambs. The highest value per ewe lamb was $6.53 and the lowest value $6, as compared with $7.28 and $6.34 for wether lambs. The maximum value for all lambs was $6.88 and the minimum value $6.27.

The averages of all lambs showed no appreciable difference between lambs from fine-wooled ewes weighing 92 pounds and those from crossbred ewes weighing 104 pounds.

Concerning the influence of the sire, the data show that Southdown-sired lambs averaged 28 cents more in value per lamb, although 2.63 pounds lighter in weight than Hampshire-sired lambs. Shropshire-sired lambs were 3.17 pounds lighter and 14 cents less valuable than Hampshire-sired lambs.

The heavy weight of both wether and ewe lambs sired by Hampshire rams and out of fine-wooled ewes was an advantage, but a correspondingly high value per lamb was not realized on account of lack of fatness.

Rank according to value per lamb of all lots of lambs differing in sire or dam was as follows: Southdown × crossbred, Hampshire × fine wool, Southdown × fine wool, Shropshire × crossbred, Hampshire × crossbred, and Shropshire × fine wool.

The influence of the sire, regardless of the dam, resulted in the following rank according to value per lamb: Southdown, Hampshire, and Shropshire.

CONCLUSIONS AND SUMMARY

In view of the data obtained in comparing Hampshire and Southdown breeding ewes, it seems reasonable to conclude that mutton-type ewes of different breeds differing markedly in body weight consume feed approximately in proportion to body weight when grazing on good pasture, or when full-fed appropriate rations for normal functions. On the other hand, lambs fed a maintenance ration, or those full-fed for rapid growth and fattening, consume feed on the basis of approximately the three-fourths power function of body weight.

Single lambs of Hampshire and Southdown breeds require total digestible nutrients, including yearly feed for the ewes, in approximately equal quantities per unit weight of Choice grade market lamb. Comparatively larger quantities of feed during the longer dry period are required by Hampshire ewes, whereas larger total quantities over

a longer time are required by the Southdown nursing ewes and growing lambs. However it seems possible that under usual farm practices, early Hampshire lambs being pushed for the June market may require relatively larger quantities of grain before the pasture season, whereas Southdown lambs may make relatively greater use of spring and summer pasture.

A twin lamb of the large early-maturing Hampshire breed requires total digestible nutrients, including its half of the ewe's yearly feed, in approximately two-thirds the quantity necessary for a similar Choice grade single lamb, or for a similar market weight of Choice grade single lamb of the small Southdown breed.

Southdown lambs of Choice market finish give higher yields of dressed carcass, boneless meat, and separable lean meat than do Hampshire lambs similar in finish; and thus are worth a higher price as live lambs, in the carcass, and in the retail trade.

Approximately a 120-percent Hampshire lamb crop, or one pair of twins to four single lambs, is necessary to produce lean meat from a carcass of Choice finish as efficiently, with respect to total digestible nutrients, as a crop of Southdown single lambs.

It may be assumed that some twins among Southdowns will tend to reduce feed costs of production, as was true among Hampshires.

Well-fed Hampshire single lambs attain Choice market finish approximately 6 weeks younger than Southdown single lambs or Hampshire twin lambs.

Southdown live lambs on a discriminating market should have as great an advantage in price per pound owing to the higher yield of boneless meat as Hampshire lambs that mature early in the season when prices are higher, and a greater advantage than late-marketed Hampshire lambs.

Area of loin eye muscle is a fairly good index of the lean meat content of the lamb carcass.

The color of lean is similar in Southdown and Hampshire Choice lamb carcasses.

The content of dry matter, total nitrogen, and ether extract is similar in boneless meat of Hampshire and Southdown lambs of Choice market grade.

The efficiency of digestion in grade lambs sired by large and small breeds, and fed on maintenance or slightly above maintenance rations, is unaffected by the breed of the sire or by the weight of the lamb. The data on purebred lambs of large and small breeds, when on full feed and about 1 year of age, indicate greater efficiency in the digestion of protein by those of the larger breed.

Market finish is a major factor in determining grade, and is important along with market weight and time of marketing in determining the value of a lamb.

There is little difference between fine-wooled and crossbred type western white-faced ewes such as were used in this experiment, either in weight or value of lamb produced when the ewes were mated with mutton-type rams. If feed consumption by the ewes is approximately in proportion to body weight, as was found to be true in comparing purebred Hampshire and Southdown ewes, the heavier crossbred ewes may be less efficient in respect to feed consumption than the fine-wooled ewes.

The small breed of sheep, Southdowns, compares favorably with the large breed of sheep, Hampshires, for economical production of market lambs in purebred flocks of the two breeds; while the use of Southdown rams with western ewes seems to be fully as desirable as the use of Hampshire rams under Central States conditions and with Federal grading of the carcasses.

Inheritance of size by the offspring is more obviously different between Hampshire and Southdown rams when they are mated with purebred ewes of the respective breeds than when they are mated with grade western ewes.

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EFFECT OF ALGAE IN RELATION TO AERATION, LIGHT, AND SOURCES OF PHOSPHORUS ON GROWTH OF TOBACCO IN SOLUTION CULTURES[1]

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INTRODUCTION

It is generally recommended that light be excluded from the roots of plants grown in solution cultures, the assumption being that this prevents the development of algae that compete with the plant. It has been shown that tobacco (*Nicotiana tabacum L.*) plants grown in solution cultures give a decided response to aeration when the solution is bubbled with compressed air.[2]

There are very few references in the literature to the effects of light on the root growth of plants in solution cultures, and only one has come to the attention of the writers relating to what is usually a concomitant effect of the admission of light to the roots, namely, the growth of green algae in the solution. Knight,[3] studying the response of plants in water cultures to aeration of the roots, used sprigs of the aquatic herb *Elodea canadensis* Michx. as one method of aeration. He found that aeration by *Elodea* had a beneficial effect on the growth of maize, but that this effect was soon obscured by the growth of algae in the solution, which appeared to do more effectively what *Elodea* had done. There was increased root growth in solutions in which algae had multiplied. No weights of roots were taken, but it was concluded from examination that the increase in growth was much too large to be accounted for by the algae alone.

The results of the preliminary experiment described below led the writers to attempt to determine quantitatively the differences that might be expected and other effects that might be observed. Three experiments were made, the results of which are reported herein.

MATERIAL AND METHODS

The stock solutions, which were diluted 20 times for growing plants, are shown in table 1. The experimental procedures were in general those described in previous publications.[4] [5] Any differences in treatment employed will be mentioned in connection with the presentation of results.

[1] Received for publication January 22, 1940.

[2] MCMURTREY, J. E., JR. THE EFFECT OF BORON DEFICIENCY ON THE GROWTH OF TOBACCO PLANTS IN AERATED AND UNAERATED SOLUTIONS. Jour. Agr. Res. 38: 371-380, illus. 1929.

[3] KNIGHT, R. C. THE RESPONSE OF PLANTS IN SOIL- AND IN WATER-CULTURE TO AERATION OF THE ROOTS. Ann. Bot. [London] 38: 305-325, illus. 1924.

[4] See footnote 2.

[5] MCMURTREY, J. E., JR. DISTINCTIVE EFFECTS OF THE DEFICIENCY OF CERTAIN ESSENTIAL ELEMENTS ON THE GROWTH OF TOBACCO PLANTS IN SOLUTION CULTURES. U. S. Dept. Agr. Tech. Bul. 340, 43 pp., illus. 1933.

TABLE 1.—*Volume-molecular concentrations of chemically pure salts used in preparing stock solutions*

Solution		Volume-molecular concentration of—					
Name	Designation	$Ca(NO_3)_2$	$CaH_4(PO_4)_2$	$CaHPO_4$	$Ca_3(PO_4)_2$	$CaCO_3$	KNO_3
Monopotassium phosphate	C_1	0.1235					0.0217
Monocalcium phosphate	P_1	.1020	0.0211				.0637
Dicalcium phosphate	P_2	.0813		0.0422			.0637
Tricalcium phosphate	P_3	.1024			0.0211		.0637
Calcium carbonate and ammonium nitrate	C_2		.0052			0.1180	
Calcium carbonate and no added nitrogen	N		.0052			.1180	

Solution		Volume-molecular concentration of—						
Name	Designation	KH_2PO_4	$Mg(NO_3)_2$	$MgSO_4$	NH_4NO_3	NH_4Cl	K_2HPO_4	$MgCl_2$
Monopotassium phosphate	C_1	0.0422	0.0124	0.0125		0.0280		
Monocalcium phosphate	P_1			.0125	0.0266			0.0141
Dicalcium phosphate	P_2		.0124	.0125	.0170	.0280		
Tricalcium phosphate	P_3			.0125	.0266			.0141
Calcium carbonate and ammonium nitrate	C_2			.0125	.1600		0.0320	.0141
Calcium carbonate and no added nitrogen	N			.0125			.0320	.0141

EXPERIMENTAL DATA

PRELIMINARY EXPERIMENT

In the spring of 1937, 20 tobacco plants of the Connecticut Broadleaf variety were put into twenty 2-quart glass fruit jars containing a complete nutrient solution (C_1). Ten of these jars were covered with several thicknesses of manila paper, while 10 had no covering of any kind and were exposed to full sunlight. In a week or 10 days it was observed that the plants in the uncovered jars were making new root growth and new top growth much more rapidly than those in the covered jars. The uncovered jars all had a thin film of algae on their sides and bottoms in addition to many algae floating throughout the solution.

Later it was observed that the roots of the plants in the uncovered jars grew down to the bottom of the jars, while those in the covered jars grew short and were bunched near the surface of the solution.

After growing about a month longer, the roots and tops were measured and the plants were harvested and air-dried. Total measurements and weights are shown in table 2.

TABLE 2.—*Average measurements and air-dry weights of tobacco plants grown in nutrient solution in covered and uncovered containers*

Condition of containers	Plants	Length of roots	Height of plant	Air-dry weight		
				Tops	Roots	Total
	Number	*Centimeters*	*Centimeters*	*Grams*	*Grams*	*Grams*
Covered	10	5	47	10.92	1.81	12.73
Uncovered	10	31	69	20.85	2.45	23.30

TESTS IN COVERED AND UNCOVERED ROOT CONTAINERS WITH THREE CALCIUM SALTS OF PHOSPHORUS

Since the results of the preceding experiment were rather striking, it was decided to carry out a greatly extended and controlled experiment and use three different nutrient solutions. There were 40 plants in each solution. One-half of the plants in each solution had been previously disinfected by treatment with silver nitrate ($AgNO_3$), 1 to 1,000; 1 group of each of the 2 lots was aerated artificially by means of compressed air; one-half of the plants of each of the four groups thus formed were put into uncovered 2-quart glass fruit jars, and the other half into covered jars. These were so arranged that all possible combinations were obtained, thus:

- Monocalcium phosphate nutrient solution (P_1)
  - Covered
    - Unaerated
      - Roots not disinfected
      - Roots disinfected
    - Aerated
      - Roots not disinfected
      - Roots disinfected
  - Uncovered
    - Unaerated
      - Roots not disinfected
      - Roots disinfected
    - Aerated
      - Roots not disinfected
      - Roots disinfected

Hence, there were 8 combinations of 5 plants each in each of the 3 nu trient solutions. All nutrient solutions were the same except for the source of phosphorus, which in the second group was supplied as dicalcium phosphate (P_2) and in the third group as tricalcium phosphate (P_3).

Before the plants were placed in the experimental solutions, they were allowed a start of about 2 weeks in dicalcium phosphate solution in uncovered, unaerated jars. At the time of transfer to the new solution (October 5) the plants not disinfected had made considerably more root and top growth than those disinfected with silver nitrate. All roots were rinsed with distilled water before the plants were placed in the new solution. Each culture was inoculated with 10 cc. of a suspension of green algae. Boron, manganese, and iron (as ferric citrate) were added at this time.

On October 18 and again on October 28 all solutions were completely renewed. On November 4, 100 cc. of stock nutrient solution was added to each culture, and on November 12 all solutions were completely renewed for the last time. The plants were harvested on November 22.

The following algae [6] were found: *Protococcus viridis* var. *infusionum* Rab., *P. viridis* var. *angulosa* Menegh., *Ulothrix parietina* (Vauch.) Kg., *Euglena viridis* Ehrb., *Chroococcus turgidis* Naeg., and *Aphanocapsa virescens* Rab. *P. viridis angulosa* occurred in greatest abundance. These are all unicellular green algae with the exception of *U. parietina*, a filamentous green alga that Dr. Chase states is often found growing with *Protococcus*. Those examined from the monocalcium phosphate cultures were greener and the cells were larger than those from cultures supplied phosphorus as dicalcium phosphate. The algae from cultures that were supplied with phosphorus from tricalcium phosphate were not examined.

[6] An examination of the algae growing in these solutions was made by Dr. Florence Meier Chase, of the Division of Radiation and Organisms, Smithsonian Institution.

In table 3 are shown the average measurements, oven-dry weights, and percentage of leaf, stalk, and root of the plants grown in nutrient solutions P_1, P_2, and P_3 respectively. Since the differences between the various treatments in solutions P_1 and P_2 are nearly parallel, the data from solution P_2 will be discussed as representative.

TABLE 3.—*Average measurements, oven-dry weights, and percentages of leaf, stalk, and roots of tobacco plants grown in various nutrient solutions*

Treatment	Height	Length of roots	Oven-dry weight				Distribution of oven-dry weight		
			Leaf	Stalk	Root	Total	Leaf	Stalk	Root
MONOCALCIUM PHOSPHATE (P_1)									
	Centimeters	*Centimeters*	*Grams*	*Grams*	*Grams*	*Grams*	*Per cent*	*Per cent*	*Per cent*
Roots disinfected:									
Uncovered, aerated	94	58	15.76	9.19	2.90	27.85	56.6	33.0	10.4
Covered, aerated	109	50	21.05	14.08	3.81	38.94	54.1	36.2	9.8
Uncovered, unaerated	88	37	12.21	9.88	3.36	25.45	47.9	38.8	13.2
Covered, unaerated	64	31	5.96	4.62	1.38	11.97	49.8	38.6	11.6
Roots not disinfected:									
Uncovered, aerated	118	46	17.81	15.83	4.84	38.49	46.3	41.1	12.6
Covered, aerated	135	37	20.31	19.11	5.1	44.56	45.5	42.9	11.5
Uncovered, unaerated	114	32	13.82	13.42	4.72	31.96	43.3	41.9	14.8
Covered, unaerated	91	28	10.08	8.39	3.12	21.59	46.7	38.9	14.4
DICALCIUM PHOSPHATE (P_2)									
Roots disinfected:									
Uncovered, aerated	104	55	15.92	11.22	3.98	31.12	51.1	36.1	12.8
Covered, aerated	121	47	20.25	15.96	4.36	40.57	49.9	39.3	10.7
Uncovered, unaerated	95	32	12.87	9.54	3.66	26.07	49.4	36.6	14.0
Covered, unaerated	39	27	3.37	1.68	.73	5.78	58.3	29.1	12.6
Roots not disinfected:									
Uncovered, aerated	125	45	20.29	16.0	5.76	42.05	48.2	38.1	13.7
Covered, aerated	119	35	19.01	18.62	4.59	42.22	45.0	44.1	10.9
Uncovered, unaerated	105	28	12.96	12.0	4.25	29.21	44.4	41.1	14.5
Covered, unaerated	81	27	8.36	9.28	2.65	20.29	41.2	45.7	13.1
TRICALCIUM PHOSPHATE (P_3)									
Roots disinfected:									
Uncovered, aerated	90	59	15.39	10.0	4.83	30.22	50.9	33.1	16.0
Covered, aerated	111	53	15.86	16.46	5.38	37.70	42.1	43.6	14.3
Uncovered, unaerated	48	44	8.41	5.21	3.12	16.74	50.2	31.2	18.6
Covered, unaerated	53	29	5.56	4.12	1.28	10.96	50.8	37.6	11.6
Roots not disinfected:									
Uncovered, aerated	114	49	17.74	16.50	7.20	41.44	42.8	39.8	17.4
Covered, aerated	128	37	17.20	19.65	5.92	42.77	40.2	46.0	13.8
Uncovered, unaerated	87	31	11.49	9.85	4.75	26.09	44.0	37.8	18.2
Covered, unaerated	67	28	6.44	6.62	2.24	15.30	42.1	43.3	14.6

Examination of the data shows that the most outstanding difference lay between the aerated and unaerated plants in covered jars. In the series in which the roots were disinfected the average weights were 40.57 and 5.78 gm., or a ratio of 7 to 1. In the corresponding series in which the roots were not disinfected, the average weights were 42.22 and 20.29 gm., or a ratio of 2.1 to 1. The smaller difference in the latter case is possibly due to the fact that the roots that were not disinfected made considerably more growth before being placed in the experimental solutions, so that the initial effect was sufficient to allow these plants to make fair growth even after being placed in unaerated solutions.

The difference between the plants that were disinfected and those that were not disinfected in the covered and aerated cultures, i. e.,

between 40.57 and 42.22 gm., respectively, while not great, probably also indicates the effect of this initial advantage. This advantage, however, was much greater at the start than at the end of the experiment, and might have been reversed had the plants been allowed to reach full maturity before harvesting. The same advantage was evident in the monocalcium and tricalcium phosphate solutions. That most of this gain was in stalk rather than leaf is shown by the relative percentages of these parts to total dry weight. In plants not disinfected the stalk represents 44 percent of the total weight as against 39 percent for disinfected plants, while the leaves of the plants that were not disinfected comprised 45 percent of the total weight as against 50 percent for the disinfected plants. The relatively greater proportion of stalk in plants not disinfected was apparently due to the well-developed and ripening seed capsules, which in disinfected plants were just forming at the time of harvesting.

Examination of the data for the uncovered cultures, and hence those in which green algae made abundant growth, shows the results of the small preliminary experiment to be further confirmed. The average weight of the plants in uncovered, unaerated jars was 26.07 gm., and that of the covered, unaerated cultures was 5.78 gm., a ratio of 4.5 to 1. Here the difference, while not as pronounced as that between covered aerated and covered unaerated plants, is still striking, and shows the effect of the algae in aerating the solution by the release of minute bubbles of oxygen. In full sunlight these bubbles were observed rising rapidly.

Taken as a whole, the roots of the plants in uncovered jars averaged a higher percentage of the total dry weight than those in covered jars, and this difference was apparently due to the weight of the algae on the roots. The actual difference of weight due to algae could not, of course, be determined.

A comparison of the plants in covered and uncovered jars, where both were aerated with compressed air, shows differences in favor of the covered cultures, though not nearly as great as the differences shown above. The plants with roots disinfected (fig. 1) show a ratio of 1.3 to 1 in favor of the covered jars, while there was practically no difference between the two groups with roots not disinfected. Here again, however, the percentage of leaf weight to total weight was greater in the case of disinfected plants, averaging 50 percent as against 42.8 percent for plants not disinfected. A greater percentage of stalk for the plants that were not disinfected again indicates a greater degree of maturity, with more weight in the maturing seed head.

One rather striking and unexpected result of the experiment was the relatively good growth made by the plants where tricalcium phosphate was used as the source of phosphorus (table 3). It is known that this form is almost insoluble in water, and in previous water-culture experiments the results with it were poor. Nevertheless, in this experiment the 10 plants in covered and aerated solutions (fig. 2) had an average dry weight of 40.24 gm., or practically the same as those in the dicalcium phosphate solution; while the 10 in uncovered aerated solutions averaged 35.83 gm. Of the latter, the untreated half averaged 41.44 gm., which is among the highest weights attained. Apparently the roots of these untreated plants had made enough growth before being placed in the experimental solutions to come into direct contact with the tricalcium phosphate

on the bottom of the jars, and were thus able to take up soluble phosphorus.

The unaerated plants (fig. 3) made much less growth than the aerated, but here again, as in the case of dicalcium phosphate, the plants in uncovered jars made much better growth than those in covered jars. In the case of the disinfected plants, those in uncovered solutions averaged 16.74 gm. and those in covered jars averaged 10.96 gm. Where the roots were not disinfected, the plants in uncovered jars averaged 26.09 gm. and those in covered jars averaged 15.30 gm. This advantage in favor of the plants that were not disinfected is greater than in the case of either monocalcium or dicalcium phosphate and is believed to be due to the same cause mentioned previously; namely that the greater initial length of the roots that were

FIGURE 1.—Tobacco plants placed in P_1 culture solution October 5, 1937; roots disinfected with silver nitrate 1 to 1,000 dilution (photographed November 6, 1937; scale shown in inches): *A*, solution darkened and aerated; *B*, light admitted to solution, which was aerated; *C*, solution darkened and unaerated; *D*, light admitted to unaerated solution.

not disinfected at the time of transfer to the new solutions brought them into direct contact with the tricalcium phosphate almost from the start.

Further study of the data for tricalcium phosphate shows that the 10 covered and aerated cultures made much better growth than the 10 uncovered and unaerated, these weights averaging 40.24 and 21.41 gm., respectively. This might indicate that the natural aeration of the nutrient solution by algae is not, of itself, sufficient for the needs of the plant. However, it was observed that in the solutions aerated through the medium of algae alone the growth of algae on the roots and on the sides of the jars was very much greater than in those cultures which, in addition to aeration by algae, were also aerated artificially. It is possible that this check upon the growth of the algae by artificial aeration was due to the mechanical agitation of the solution; it is also possible that it was due to a changed chemical balance within the solution caused by artificial aeration. The same phenomenon was observed in the monocalcium and dicalcium phosphate solutions, but it was not so pronounced. At any rate, it

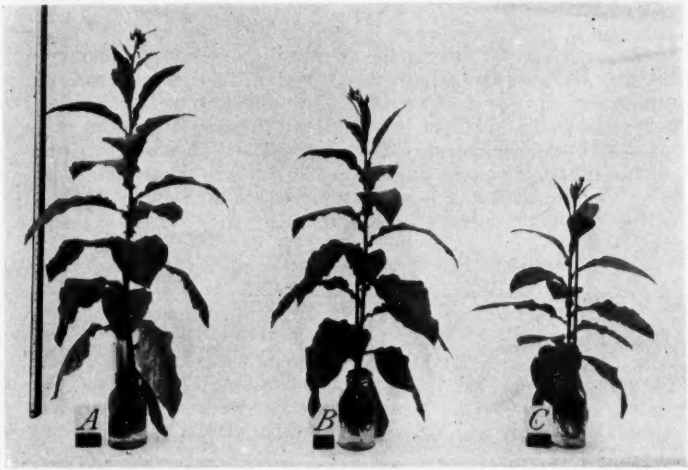

FIGURE 2.—Tobacco plants placed in culture solution October 5, 1937; roots not disinfected (photographed November 6, 1937; scale shown in inches). Light admitted to unaerated culture solutions, with phosphorus derived from different sources: *A*, Monocalcium phosphate (P_1); *B*, dicalcium phosphate (P_2); *C*, tricalcium phosphate (P_3).

FIGURE 3.—Tobacco plants placed in culture solution October 5, 1937 (photographed November 6, 1937; scale shown in inches). Culture solutions darkened, with phosphorus derived from different sources: *A*, Monocalcium phosphate (P_1); *B*, dicalcium phosphate (P_2); *C*, tricalcium phosphate (P_3).

is apparent that up to a certain point aeration by algae is definitely beneficial to the plant; but when the algae, for whatever reason, become too abundant, actual competition for the available nutrients robs the growing plant. It is also probable that too great an accumu-

lation of algae upon the roots may result in partly stopping the intake of nutrients.

If the average weights of the plants in covered and uncovered cultures are observed as a whole, regardless of the other treatments, they compare as shown in table 4. The uncovered cultures are thus shown to have made better growth in all three solutions, on the whole, than the covered cultures. This difference was greatest in the case of dicalcium phosphate, amounting to about 18 percent. The uncovered monocalcium solutions showed an increase of 5.7 percent, and the tricalcium solutions 7.3 percent.

TABLE 4.—*Average dry weight of tobacco plants as affected by the various treatments considered separately*

Treatment	Plants in culture solution	Monocalcium phosphate (P_1)	Dicalcium phosphate (P_2)	Tricalcium phosphate (P_3)
	Number	*Grams*	*Grams*	*Grams*
Covered	20	29.27	27.21	26.68
Uncovered	20	30.94	32.11	28.62
Aerated	20	37.46	38.99	38.03
Unaerated	20	22.75	20.34	17.27
Aerated by algae	10	28.71	27.64	21.41
Unaerated	10	16.78	13.04	13.12

The advantage of aerated over unaerated solutions, regarded as a whole, is shown by the total weights in table 4.

Here the increase due to aeration is very great, amounting to 64.7 percent for monocalcium, 91.7 percent for dicalcium, and 120.2 percent for tricalcium phosphate solutions. The very great increase in growth with aeration in the case of tricalcium phosphate emphasizes again its effect in causing the roots to grow down into direct contact with this compound.

It may be argued that distinction should be made between the cultures that were aerated both naturally and artificially (i. e., by algae and compressed air together) and those that were aerated by algae alone. Such comparison between the cultures aerated by algae alone and those not aerated at all is shown in table 4. Here the increase due to algae alone over unaerated cultures was 71.1 percent for monocalcium, 111.9 percent for dicalcium, and 63.2 percent for tricalcium phosphate. The average increase in this case was 82.1 percent, as compared with an average increase of 92.2 percent in the aerated cultures.

REACTION OF NUTRIENT SOLUTIONS[7]

In order to determine to what extent the pH value of the nutrient solutions was changed during the course of the experiment, representative samples of the solutions were taken at the time of harvesting. These were tested by the quinhydrone electrode method. To obtain a basis for comparison, freshly made solutions of each of the three basic nutrient solutions were tested at the same time. The pH readings of the various solutions are shown in table 5.

[7] Determinations of reaction were performed by G. E. Halliday, assistant biochemist, Division of Tobacco and Plant Nutrition.

TABLE 5.—*pH values of nutrient solutions when freshly made and at time of harvesting*

Solution and treatment	Monocalcium phosphate (P_1)	Dicalcium phosphate (P_2)	Tricalcium phosphate (P_3)
	pH	*pH*	*pH*
Freshly made solution	4.2+	4.9	4.9+
Solution at time of harvesting:			
Roots disinfected:			
Uncovered, aerated	5.8	5.9	6.7
Covered, aerated	5.6	6.1	6.5
Uncovered, unaerated	6.8	6.9	6.8
Covered, unaerated	5.9	6.2	6.5
Roots not disinfected:			
Uncovered, aerated	6.1	6.1	7.0
Covered, aerated	6.0	6.2	6.7
Uncovered, unaerated	6.8	6.7	6.6
Covered, unaerated	6.4	7.0	6.9

It will be observed that the pH values of the freshly made nutrient solutions tend to approach what is generally considered the optimum for tobacco.[8] These low pH values were temporary, however, for the readings taken at the time of harvesting all show a marked rise in their pH value.

In order to learn whether there was any correlation between the growth of the algae and the hydrogen-ion concentration of the solutions, the covered and uncovered jars were compared in pairs through each series. That is, an uncovered aerated solution was compared with a covered aerated solution, and an uncovered, unaerated solution was compared with a covered, unaerated solution. By this method of comparison, the uncovered solutions, i. e., those containing algae, in monocalcium phosphate were shown to have a higher pH value throughout. However, in the case of dicalcium phosphate, only one out of four pairs showed a higher pH value for the uncovered cultures, while in the case of tricalcium phosphate, three out of four showed a higher pH value in uncovered jars. The cultures were then compared, within each kind of solution, to learn whether there was any correlation between the hydrogen-ion concentration and artificial aeration. By this comparison, it can be seen that every aerated culture had a lower pH value than its corresponding unaerated culture, except in one case when they were the same: The uncovered aerated cultures in the disinfected group with tricalcium phosphate had a pH value of 7.0, the highest attained, as compared with a pH value of 6.6 for the corresponding unaerated cultures. Further examination (table 3) shows that these aerated cultures had a greater dry weight than their corresponding unaerated cultures.

Apparently, then, there is little correlation between the growth of algae and the hydrogen-ion concentration of the solution, and no close correlation between the growth of the tobacco plant and the hydrogen-ion concentration of the solution. The fact that none of the pH readings in this experiment was equal to or lower than that of the freshly made nutrient solution may have been due to the continuous presence of enough calcium ions to keep the pH value fairly high.

[8] ANDERSON, P. J., OSMUN, A. VINCENT, and DORAN, W. L. SOIL REACTION AND BLACK ROOT ROT OF TOBACCO. Mass. Agr. Expt. Sta. Bul. 229, pp. 118–136, illus. 1926.

During the latter part of the experiment it was observed that some of the roots showed root rot. This is assuredly not new in experiments with water cultures, but the interesting feature was that all of these plants, or practically all, were in covered jars. At the time of harvesting, notes were taken on these roots and their number tabulated as to treatment (table 6).

TABLE 6.—*Number of tobacco plants with defective roots, with their respective treatments*

Treatment	Plants with defective roots	Reaction of solution
	Number	*pH*
Monocalcium phosphate; covered, aerated, not disinfected	1	6.0
Dicalcium phosphate; covered, aerated, not disinfected	2	6.2
Dicalcium phosphate; covered, unaerated, not disinfected	2	7.0
Tricalcium phosphate; covered, aerated, disinfected	1	6.5
Tricalcium phosphate; covered, aerated, not disinfected	1	6.7
Tricalcium phosphate; uncovered, unaerated, disinfected	1	6.8

It will be noted at once that, of the eight plants with defective roots, seven were in covered jars. It is true that six of the eight had not been disinfected with silver nitrate, thus increasing the chances for infection. Nevertheless, the fact that only one plant of those growing in uncovered jars showed infection, as compared with seven in the covered jars, appears to indicate a definite protective effect on the part of the light or the algae growing on the roots, or possibly both. Whether this was purely mechanical and due to the covering of algae on the roots, or partly mechanical and partly chemical could not be determined. It is possible, however, that the effect was partly chemical, for the roots of the great majority of the plants in uncovered jars were not completely covered with algae. These roots or parts of roots that to the unaided eye showed no film of algae were a pure, shining pearl white, in contrast to many of those in covered cultures, which, especially as they approached maturity, assumed a dull, yellowish tinge. Some of the algae-covered rootlets were examined under the microscope in order to learn whether there was any symbiotic relationship such as exists between mycorrhiza and the roots of certain trees. No such relationship was evident.

TESTS WITH BLUE-GREEN ALGAE

In February 1938 a further experiment was begun, the object of which was twofold: (1) To learn whether the results of the preceding experiment would be verified; and (2) to find whether certain species of blue-green algae could, in the absence of nitrogen, fix enough atmospheric nitrogen in the nutrient solution to appreciably affect the growth of tobacco plants. Two nutrient solutions were used, one with nitrogen added (C_2) and the other with nitrogen withheld (N).

The cultures were arranged in groups of five plants each, as in the preceding experiment. Five cultures inoculated with blue-green algae were matched with five under similar conditions of exposure to light and aeration but inoculated with green algae. Of the latter, three

in each group were inoculated with 10 cc. each of an infusion of unicellular algae, and two with 10 cc. each of an infusion of filamentous algae.

The species of blue-green algae used was *Nostoc muscorum* Ag.,[9] which is capable of more rapid nitrogen fixation than other known species of blue-green algae.[10] For that reason it was chosen for this experiment. Allison found that this species was able to fix 18 to 30 mg. of nitrogen per 100 cc. of solution when a small amount of glucose was added. Its nitrogen-fixing ability is greatly increased when glucose is added. However, glucose was not used in this experiment.

On February 6 the experiment was set up. All jars, stoppers, glass tubes, and rubber hose were steam-sterilized. One hundred and twenty plants of the Connecticut Broadleaf variety, carefully selected for uniformity, were washed and then disinfected with silver nitrate, 1 to 1,000, the roots for 5 minutes and the tops for 2 minutes. Each nutrient solution was arranged as shown below:

- Blue-green algae
  - Covered
    - Aerated
    - Unaerated
  - Uncovered
    - Aerated
    - Unaerated
- Green algae
  - Covered
    - Aerated
    - Unaerated
  - Uncovered
    - Aerated
    - Unaerated
- Control (no algae)
  - Covered
    - Aerated
    - Unaerated
  - Uncovered
    - Aerated
    - Unaerated

The culture of *Nostoc* as received was in the form of large, thin, but somewhat tenacious films. In order to supply these to the nutrient solutions in as nearly equal quantities as possible, the *Nostoc* was well shaken up with sterilized glass beads. This procedure broke up the films very satisfactorily. Each culture to receive *Nostoc* was then inoculated with 10 cc. of this infusion. Iron and manganese were added to all cultures, and on April 2, 2 cc. of zinc sulfate solution was added to each culture, as several were beginning to show signs of what appeared to be zinc deficiency. On April 11 all of the nitrogen cultures were completely renewed. Measurements of height and length of roots were taken at biweekly intervals throughout the growing period. On May 11 the plants were harvested and hung for air-drying.

The measurements and air-dry weights of the plants in cultures to which ammonium nitrate was added are shown in table 7.

[9] Supplied as a pure culture by F. E. Allison, of the Fertilizer Research Division, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

[10] ALLISON, FRANKLIN E., HOOVER, SAM R., and MORRIS, HERMAN J. PHYSIOLOGICAL STUDIES WITH THE NITROGEN-FIXING ALGA, NOSTOC MUSCORUM. Bot. Gaz. 98: 433–463, illus. 1937.

TABLE 7.—*Average measurements during growth period and air-dry weights of tobacco plants grown in nutrient solution with added nitrogen, with and without green algae and the blue-green alga Nostoc muscorum*

Treatment	Average height of plants					Average lengths of roots					Average air-dry weight per plant			
	Mar. 4	Mar. 18	Mar. 31	Apr. 15	Apr. 29	Mar. 4	Mar. 18	Mar. 31	Apr. 15	Apr. 29	Roots	Leaves	Stalks	Total
Covered:	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
G,[1] unaerated	5	6	9	19	33	11	12	13	14	15	1.20	3.44	2.48	7.12
B,[1] unaerated	5	6	8	19	32	12	15	17	16	16	1.37	3.70	2.48	7.55
G, aerated	4	5	13	34	73	12	15	22	28	33	3.70	12.17	12.12	27.99
B, aerated	4	6	15	42	85	12	17	25	32	35	4.32	12 18	14.25	30.75
Control, unaerated	5	7	11	23	32	13	15	16	16	16	.89	2.47	2.48	5.84
Control, aerated	4	5	17	45	90	11	11	32	35	35	5.29	12.43	16.73	34.45
Uncovered:														
G, unaerated	4	7	14	28	58	9	10	28	34	36	5.18	9.51	9.39	24 08
B, unaerated	5	8	20	45	61	11	21	29	34	35	4.17	8.16	8.90	21.23
G, aerated	4	4	4	6	17	8	9	8	10	28	2.57	8.26	4.19	15.02
B, aerated	4	6	6	7	22	8	9	8	13	31	3.59	8.87	5.71	18.17
Control, unaerated	5	7	15	38	62	12	15	27	33	36	4.24	7.76	11.60	23.60
Control, aerated	4	4	5	7	25	8	9	9	12	28	2.50	8.05	5.05	15.60

[1] G represents green algae; B represents blue-green algae.

In this series, an attempt was made to prevent the uncovered cultures inoculated with *Nostoc* from being contaminated by green algae from the air. Glass wool was packed about the stems of the plants and into the other holes in the corks, but it proved inadequate for the purpose. Green algae were soon observed in these cultures, and in a short time became as abundant as those in the jars that had been originally inoculated with green algae; thus any effects that might have been due to *Nostoc* were wiped out or rendered unobservable. There were no significant differences between the cultures with unicellular and those with filamentous green algae, and therefore they will not be dealt with separately.

Study of the data shows that the greatest difference again lay between the aerated and unaerated plants (fig. 4) in covered jars. The plants in the covered, aerated jars averaged 31.06 gm. and those in the covered, unaerated jars 6.84 gm., a ratio of 4.5 to 1. This agrees with the results of the previous experiment, although the difference is not as large as in the latter. This is no doubt due to the fact that in the first experiment the nutrient solutions were renewed three times, while in this experiment there was only one renewal.

A comparison of uncovered, unaerated cultures with the covered, unaerated cultures shows an average weight of 22.97 gm. for the uncovered as compared with 6.84 gm. for the covered jars, a ratio of 3.3 to 1. This confirms the effect observed before, namely, the beneficial effect of aeration by algae on the growth of the plant.

In comparing the uncovered, unaerated with the uncovered, aerated cultures, there appears to be a discrepancy between the results in this experiment and those recorded in the previous experiment. In this experiment the unaerated plants (fig. 5) made better growth than those aerated, the average weights being 22.97 and 16.26 gm. respectively, a ratio of 1.4 to 1. In the previous experiment, the ratio (with the dicalcium phosphate solution) was 1.3 to 1, but with the advantage in favor of the aerated cultures. This apparent discrepancy can be explained by examination of the rate of growth of the plants in uncov-

ered, aerated cultures, as revealed in the record of height and length of roots throughout the period of the experiment. These data show that the plants remained practically at a standstill until the early part of April, but the measurements of April 15 show a marked increase, which was accelerated within the remaining 2 weeks the plants were

FIGURE 4.—Tobacco plants placed in culture solution (C_2) February 6, 1938 (photographed May 4, 1938): *A*, Culture solution darkened and aerated; *B*, culture solution darkened, unaerated.

allowed to grow. Until these plants started normal growth, they exhibited the familiar symptoms of iron chlorosis, and this in spite of the fact that iron was added to these cultures at the rate of 2 cc. of solution per day for a period of 2 weeks. It is possible that the high pH value of the solution, due to the large amount of calcium carbonate present, caused precipitation of the iron or otherwise rendered it unavailable. Although all the cultures had the high amount of calcium carbonate, it seems reasonable to suppose that, at least in

those uncovered cultures that were not aerated, the lack of agitation of the solution aided in causing the iron to remain more available, since in unagitated solutions the upper layers do not exhibit the chemical concentration that occurs in the lower layers. The fact that growth

FIGURE 5.—Tobacco plants placed in culture solution (C_2) February 6, 1938 (photographed May 4, 1938): *A*, Light admitted to aerated solution; *B*, light admitted to unaerated solution.

proceeded normally in covered cultures that were aerated indicates that there may be a light factor involved also. The conditions affecting the intake of iron by plants in solution cultures are not well understood. Under a given set of conditions the plants may start normal growth and continue without interruption; under what are apparently the same conditions, they may develop symptoms of iron chlorosis from which they may not recover for several weeks, even though iron be added every day or two. In the experience of the writers, iron chlorosis cannot be corrected within 24 to 48 hours, as has been stated

by some investigators. However, it was observed during these and other experiments that intake of iron at the start of the experiment is made more certain if the culture is not at first aerated artificially.

The results of the experiment with the blue-green alga *Nostoc muscorum* in solutions containing no nitrogen are shown in table 8.

TABLE 8.—*Average measurements during growth period and air-dry weights of tobacco plants grown in nutrient solution with no added nitrogen, with and without green algae and the blue-green alga Nostoc muscorum*

Treatment	Average height of plants					Average lengths of roots					Average air-dry weight per plant			
	Mar. 4	Mar. 18	Mar. 31	Apr. 15	Apr. 29	Mar. 4	Mar. 18	Mar. 31	Apr. 15	Apr. 29	Roots	Leaves	Stalks	Total
Covered:	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
G,[1] unaerated	5	6	7	8	8	21	35	52	68	80	0.36	1.09	0.30	1.75
B,[1] unaerated	5	6	6	7	7	24	42	48	60	77	.35	1.02	.26	1.63
G, aerated	4	5	5	6	6	17	36	54	71	77	.31	.89	.19	1.39
B, aerated	5	6	6	7	7	14	31	48	58	69	.29	.81	.20	1.30
Control, unaerated	4	5	5	6	6	30	43	52	57	64	.24	.65	.18	1.07
Control, aerated	4	4	5	5	6	23	46	59	62	71	.27	.68	.18	1.13
Uncovered:														
G, unaerated	5	5	6	7	7	33	49	63	79	91	.27	.83	.20	1.30
B, unaerated	5	5	6	6	6	30	54	65	70	76	.35	1.0	.20	1.55
G, aerated	5	7	7	8	8	47	53	67	81	99	.46	1.24	.32	2.02
B, aerated	4	5	6	6	6	41	51	58	58	63	.55	1.13	.26	1.94
Control, unaerated	4	5	6	6	6	34	46	53	64	72	.26	.75	.19	1.20
Control, aerated	4	5	5	6	7	34	55	63	69	83	.45	.91	.24	1.60

[1] G represents green algae; B represents blue-green algae.

At no time during the experiment did any of the plants in this series show evidences of normal growth. They exhibited the characteristic symptoms associated with lack of nitrogen. The plants remained small, the leaves small and yellow, with firing of the lowest leaves. Study of the measurements of height shows that in only three groups did the average increase in height exceed 2.5 cm. The roots, however, increased very greatly in length, in a number of cases reaching a length of over 100 cm. and in one case a length of 120 cm. The tendency of the roots to elongate in the absence of nitrogen has been previously reported by McMurtrey.[11] However, while the roots increased greatly in length, their total number was much less, so that their total weight was small. This is shown in the data for dry weights, in which the average weight of roots per plant ranges from about one-fourth to one-half of a gram. On a percentage basis, however, these roots average over 23 percent of the total dry weight, while those in the nitrogen series average about 12 percent. Part of this increase may have been due to the weight of *Nostoc* on the roots, but the values for the cultures without *Nostoc* show that its added weight could not have been very great. The *Nostoc* grew in greatest numbers on the bottoms of the jars, where it formed rounded, more or less hemispherical, colonies with a lacy, much-branched structure, resembling miniature sponges. In no case were the cultures observed to form thin films such as were noted in the inoculating culture. They did, however, show a definite blue-green color that had not been observable in the parent culture. No colonies were formed in covered jars, and no growth was visible to the naked eye, although Allison [12] found that *Nostoc* is able to grow in subdued light, and even, for a time, in the absence of light, provided sugar is added to the nutrient solution. No growth of green algae was detected in any of the cultures in this series, either covered or uncovered.

[1] See footnote 2.

[12] See footnote 10.

The high pH value of the nutrient solution due to the calcium carbonate used in this experiment is favorable to the growth of *Nostoc*, according to Allison. Nevertheless, although these algae made some growth, the amount of atmospheric nitrogen fixed by them was evidently too small or was not released so as to make any significant difference in the growth of the accompanying tobacco plants. In one series there is a small apparent difference in favor of the *Nostoc* cultures over the controls, but this is offset by the fact that one series of green algae cultures gave a higher yield than either *Nostoc* or the controls. The differences are therefore judged not to be significant. The average increase in dry weight, based on a comparison with 50 sample plants selected and dried at the beginning of the experiment, was 0.302 gm. for the roots, 0.893 gm. for the leaves, and 0.196 gm. for the stems, or a total of 1.39 gm. per plant.

SUMMARY AND CONCLUSIONS

Tobacco (*Nicotiana tabacum* L.) plants were grown in three different nutrient solutions, three sources of phosphorus being used. The plants were grown in aerated and unaerated solutions, with and without green algae and with roots exposed to or shielded from light, in an arrangement whereby all possible combinations of these factors were effected. The nitrogen-fixing ability of the blue-green alga, *Nostoc muscorum*, also was studied under the conditions of these experiments. The rate of growth was recorded in measurements of height of plant, length of roots, and final weights obtained after oven-drying the plants.

Green algae function as efficient aerators of nutrient solutions up to the point where their numbers offer serious competition for the available nutrients. Artificial aeration apparently acts as a check upon the growth of algae.

Artificial aeration gives the greatest increase in growth of tobacco plants in solution cultures.

Green algae associated with light appear to have a protective effect on tobacco roots against infection by fungi. The evidence indicates that this may be both physical and chemical.

There is no evidence from these experiments that the growth of green algae is correlated with the hydrogen-ion concentration of the nutrient solution.

The assimilation of iron by the plant at the beginning of a water-culture experiment may be retarded by artificial aeration.

The phosphorus in tricalcium phosphate is available to tobacco plants in solution cultures provided the roots come into direct contact with it.

The use of nutrient solutions containing green algae, exposed to light and not artificially aerated, is believed to be of value in starting plants for use in water-culture experiments. In no case where these conditions obtained, have the writers so far failed to secure uniform starting and growing plants. Once such a start is obtained, later transfer to covered, artificially aerated cultures can be made without any set-back to the plants.

Blue-green algae were not able to fix sufficient atmospheric nitrogen, in the nutrient solution used, to affect the growth of tobacco plants.

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